IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	H. William BOSCH et al.
Title:	NOVEL GLIPIZIDE COMPOSITIONS
Appl. No.:	10/701,064
Filing Date:	11/5/2003
Examiner:	Susan T. TRAN
Art Unit:	1615
Confirmation Number:	6295

DECLARATION UNDER 37 C.F.R. §1.132

The undersigned, Gary Liversidge, hereby declares as follows:

I. Background of Gary Liversidge

1. I received my Ph.D. in 1981 from the University of Nottingham, England, in Pharmaceutical Chemistry. I have been working in the field of nanoparticulate drug technology since 1987, when I joined Eastman Pharmaceuticals.

2. Through a series of business transactions, Eastman Pharmaceuticals became Sterling Winthrop Pharmaceuticals Research Division, which became known as NanoSystems. This business is currently known as the Elan Drug Technologies (EDT) business division of Elan Corp. PLC. Intellectual property developed at EDT is owned by Elan Pharma International Ltd. (an affiliate of Elan Corp., PLC), which is the assignee of the above-referenced patent application.

3. Currently I am Vice-President and Chief Technology Officer of EDT, with offices at 3500 Horizon Drive, King of Prussia, PA 19406.

II. Nanoparticulate formulations do not always improve bioavailability of the active agent in comparison to other formulations

A. U.S. Patent No. 7,217,431

4. U.S. Patent No. 7,217,431 ("the '431 patent" submitted herewith as Exhibit A) demonstrates that a nanoparticulate formulation of a drug substance according to Elan's nanotechnology does not improve *in vivo* bioavailability of the drug in comparison to other non-nanoparticulate formulations of the same drug substance. *See* Example 4, which is summarized in the following paragraphs. This data therefore demonstrates that a researcher cannot predict whether an active agent will exhibit an improved pharmacokinetic profile by reformulating the active agent into a nanoparticulate formulation.

5. Five different formulations of a drug substance, including a nanosuspension of the drug supplied by Elan (the assignee of the present application), were orally administered to dogs at similar doses and then tested for *in vivo* bioavailability. *See* column 24, line 61 through column 25, line 37. The nanosuspension of the drug comprised nanoparticulate drug (e.g., drug particles having an effective average particle size of less than 1 micron), HPC-SL as a surface stabilizer adsorbed to the surface of the drug particles, and water. The particulate material of formulation B or C had a geometric weight mean diameter between 75 μm and 2000 μm. *See* column 38, claim 1.

Table 1		
Formulations	**Contents**	**Dose**
A	Nanosuspension stabilized by hydroxyl propyl cellulose (HPC-SL) (supplied by Elan)	36.3 mg
B	Tablets containing a particulate material of the drug	37.5 mg
C	Tablets containing a particulate material of the drug	42.4 mg
D	Capsules containing a microemulsion of the drug	36.5 mg
E	Capsules containing a microemulsion of the drug	37.2 mg

6. Despite the minor variation in the dose of each formulation administered to dogs, the non-nanoparticulate tablet and capsule formulations achieved higher *in vivo* bioavailability, as represented by higher C_{max} and AUC, in comparison to the nanosuspension of the same drug substance. *See* the table spanning columns 27 and 28, the results of which are summarized in the table below.

Table 2				
Formulations	**C_{max}**		**AUC**	
	C_{max} (ng/ml)	**% relative to Formulation A**	**$AUC_{0\text{-}inf}$ (ng/ml)**	**% relative to Formulation A**
A	19 ± 8	--	206 ± 108	--
B	52 ± 15	274%	489 ± 187	237%
C	29 ± 17	153%	290 ± 184	141%
D	35 ± 13	184%	318 ± 144	154%
E	42 ± 6	221%	318 ± 65	154%

7. Accordingly, both non-nanoparticulate capsule and tablet formulations containing drug particles of much larger particle size exhibited *greater* bioavailability as compared to a nanoparticulate formulation, as demonstrated by the '431 patent.

B. Ketoprofen

8. Vergote et al. ("In vivo evaluation of matrix pellets containing nanocrystalline ketoprofen," *Int'l. J. Pharm.*, *240*: 79-84 (2002); Exhibit B), describes comparison of the bioavailability of a microparticulate ketoprofen composition and a nanoparticulate ketoprofen composition following administration to dogs. The microparticulate ketoprofen composition had a mean particle size of 65 µm, and the nanoparticulate ketoprofen composition had a mean particle size of 265 nm. *See* page 80, right column, 1st paragraph.

9. The nanoparticulate ketoprofen composition and the microparticulate ketoprofen composition demonstrated similar performance in bioavailability (represented by C_{max} and AUC) in both forms of pellets and compressed pellets, as shown in Table 1, page 83, relevant data excerpted and compared below.

Table 3

Ketoprofen Formulations		C_{max} (µg/ml)	% of difference	AUC (µg/h/ml)	% of difference
Pellets (n = 6)	Nanoparticulate	12.8 ± 0.8	25%	94.1 ± 9.5	9%
	Microparticulate	10.2 ± 1.5		86.1 ± 7.0	
Compressed pellets (n = 3)	Nanoparticulate	7.7 ± 0.3	24%	93.2 ± 6.8	2%
	Microparticulate	6.2 ± 0.4		91.4 ± 7.3	

10. Both C_{max} and AUC are required elements for bioavailability. While the maximum concentration of ketoprofen was about 24-25% higher for the nanoparticulate formulation of ketoprofen, the total quantity of drug absorbed following administration was almost identical for the nanoparticulate and microparticulate ketoprofen formulations (i.e., differences of 2% and 9%).

11. Pursuant to the accompanying FDA guidance for "Bioavailability and Bioequivalence Studies for Orally Administered Drug Products" (revision 1, March 2003;

Exhibit C), two drugs or two formulations are considered bioequivalent if the AUC and C_{max} are within 80-125%. *See* page 20, last paragraph. Thus, this reference demonstrates that formulating a drug into a nanoparticulate drug formulation does not necessarily lead to improved pharmacokinetic properties, such as bioavailability. Moreover, this reference also demonstrates that a researcher cannot predict that a nanoparticulate drug formulation will perform better than a non-nanoparticulate formulation of the same drug, based on improved pK profiles of a different drug formulated into a nanoparticulate drug formulation.

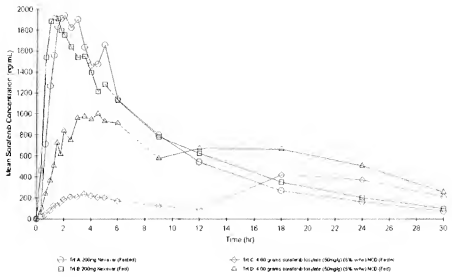
III. Nanoparticulate drug formulations do not always eliminate food effect of the drug in comparison to non-nanoparticulate formulations of the same drug

12. Nexavar® (sorafenib tosylate) is an orally bioavailable multi-kinase inhibitor containing large particles (e.g., greater than 10 microns) of the active agent, sorafenib tosylate. Nexavar® is prescribed for hepatocellular carcinoma and for patients with advanced renal carcinoma at a twice daily, 200 mg tablet.

13. A nanoparticulate sorafenib tosylate composition comprising 0.6% Tween 80, 0.07% docusate sodium, and 35% D-sorbitol was prepared. The nanoparticulate sorafenib tosylate composition had a D_{50} particle size of less than 300 nm.

14. The plasma pharmacokinetic profiles of Nexavar® and the nanoparticulate sorafenib tosylate composition were compared after each formulation was orally administered to dogs under fasted and fed conditions.

15. As demonstrated in the figure below, Nexavar® exhibited ***minimal*** variation in mean plasma concentration of sorafenib tosylate between fasted and fed dogs; whereas the nanoparticulate sorafenib tosylate composition exhibited ***significant*** variation in mean plasma concentration of sorafenib tosylate between fasted and fed dogs.



16. An elimination of food effect is represented by a fed/fasted ratio of 1.0. In other words, the closer the fed/fasted ratio to 1.0, the less food effect for the drug formulation. As shown in the table below, Nexavar® did not show much food effect as demonstrated by ***minimal*** variations in AUC, C_{max} and T_{max} between fasted and fed dogs. However, the nanoparticulate sorafenib tosylate composition exhibited ***significant*** food effect as demonstrated by big variations in AUC, C_{max} and T_{max} between fasted and fed dogs.

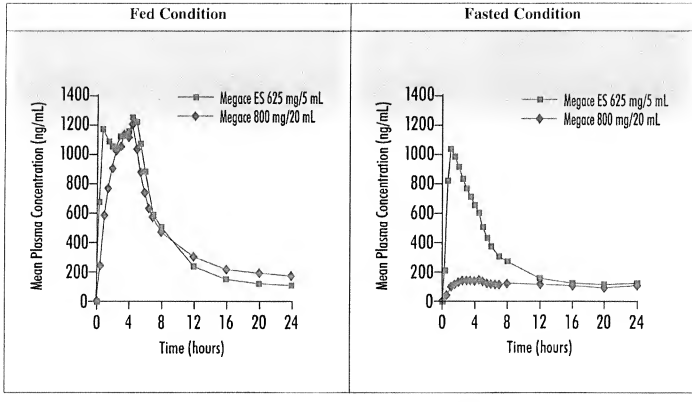
Table 4						
PK parameters	**200 mg Nexavar® tablet**			**4.0 g (50 mg/g) nanoparticulate sorafenib tosylate composition (NCD)**		
	Fasted N=6	**Fed N=6**	**Fed/Fasted Ratio**	**Fasted N=6**	**Fed N=6**	**Fed/Fasted Ratio**
AUC_{last} (ng/mL.hr)	18040.090 ± 10610.914	18986.991 ± 6399.603	1.05	7443.356 ± 7901.862	18419.494 ± 8754.795	2.47
AUC_{inf} (ng/mL. hr)	18733.619 ± 11035.52	20138.630 ± 7210.515	1.08	7432.754 ± 9673.851	19622.505 ± 10535.396	2.64
C_{max} (ng/mL)	2286.766 ± 1446.826	2170.977 ± 678.647	0.95	581.624 ± 571.488	1221.659 ± 368.175	2.1
T_{max} (hr)	1.917 ± 0.606	1.500 ± 0.548	0.78	9.250 ± 9.310	3.417 ± 1.656	0.37

IV. It is unpredictable whether a nanoparticulate formulation of an active agent achieves improved bioavailability of the active agent in the presence of food or in the absence of food

A. Megestrol Acetate – maximum bioavailability achieved in the presence of food

17. Megace® is a ***micronized*** megestrol acetate formulation having a particle size of less than 20 microns. Megace ES® is a ***nanoparticulate*** megestrol acetate formulation having a particle size of less than 200 nm and having a surface stabilizer adsorbed to the surface of the megestrol particles. *See* U.S. Patent Nos. 5,338,732 and 7,101,576, which describe the particle size information of Megace® and Megace ES®.

18. As shown in the figures below, Megace® exhibited ***significant*** variation in bioavailability when administered in fed state and in fasted state, whereas Megace ES® essentially diminished the food effect on bioavailability.



19. Nanoparticulate formulation of megestrol acetate achieved the maximum bioavailability in the presence of food. This is surprising, as other drugs demonstrate maximum bioavailability when formulated into a nanoparticulate drug formulation in the ***absence*** of food, as described in the section below.

20. This data demonstrates that at present it is not possible to predict whether a drug formulated into a nanoparticulate drug formulation will exhibit maximum bioavailability in the presence or in the absence of food.

B. Cilostazol – maximum bioavailability achieved in the absence of food

21. A ***microparticulate*** cilostazol composition having a D_{50} particle size of 2.4 microns exhibited significant variation in bioavailability when administered to fed beagle dogs and to fasted beagle dogs. However, the food effect was greatly reduced when a ***nanoparticulate*** cilostazole composition having a D_{50} particle size of 0.22 microns (or 220 nm) was administered to fed beagle dogs and to fasted beagle dogs, as shown in the table below.

Table 5

	Nanoparticulate Cilostazol Composition	Microparticulate Cilostazol Composition
D_{50} Particle Size (μm)	0.22	2.4
Fed AUC (ng·h/mL)	13589 ± 3895	10669 ± 3417
Fasted AUC (ng·h/mL)	17832 ± 4994	2875 ± 587
Fed/fasted ratio	0.76 ± 0.04	3.7 ± 0.7

22. Nanoparticulate formulation of cilostazol achieved the maximum bioavailability in the ***absence*** of food. This is surprising, as other drugs demonstrate maximum bioavailability when formulated into a nanoparticulate drug formulation in the ***presence*** of food, as described in the section above.

23. This data demonstrates that at present it is not possible to predict whether a drug formulated into a nanoparticulate drug formulation will exhibit maximum bioavailability in the presence or in the absence of food.

C. MK-0869 – maximum bioavailability achieved independent of the fed/fasted condition

24. A ***microparticulate*** MK-0869 composition having a mean particle size of 5.5 microns exhibited significant variation in bioavailability when administered to fed beagle dogs and to fasted beagle dogs. However, the food effect was essentially eliminated when a

nanoparticulate MK-0869 composition was administered to fed beagle dogs and to fasted beagle dogs, as shown in the table below.

Table 6

	Nanoparticulate MK-0869 Composition	Microparticulate MK-0869 Composition
Mean Particle Size (μm)	0.12	5.5
Fed AUC (ng·h/mL)	24385 ± 3261	18715 ± 3240
Fasted AUC (ng·h/mL)	25287 ± 3290	5883 ± 1862
Fed/fasted ratio	0.96	3.2

25. Nanoparticulate formulation of MK-0869 achieved the maximum bioavailability independent of the fed/fasted states. This is surprising, as other drugs demonstrate maximum bioavailability when formulated into a nanoparticulate drug formulation in the ***presence*** of food, while yet other drugs demonstrate maximum bioavailability when formulated into a nanoparticulate drug formulation in the ***absence*** of food, as described in the sections above.

26. This data demonstrates that at present it is not possible to predict whether a drug formulated into a nanoparticulate drug formulation will exhibit maximum bioavailability in the presence or in the absence of food.

V. Not all active agents can be formulated into stable nanoparticulate active agent compositions

A. Banavath

27. Although nanoparticulate active agent compositions may improve bioavailability of the active agent, the technologies employed to obtain nanoparticulate active agent compositions, such as precipitation, microemulsion, high pressure homogenization, and milling, are all associated with disadvantages. *See* Banavath et al., "Nanosuspension: An Attempt To

Enhance Bioavailability Of Poorly Soluble Drugs," *Int'l J. Pharm. Sci. and Res.* 1(9): 1-11 (2011) (submitted herewith as Exhibit D), at page 4, Table 2.

28. More specifically, precipitation may cause the growth of drug crystals and requires that the drug be soluble in at least one solvent. Microemulsion requires the use of a high amount of surfactant and stabilizer, which increases production cost. At times, microemulsion even involves the use of hazardous solvents in production. Homogenization requires that the drug be pre-processed into a micronized state, and possible contamination may occur from metal ions from the wall of the homogenizer. Milling is a time-consuming process which is hard to scale up and which may have contamination from the milling media. Also prolonged milling may induce instability of the drug, resulting in the drug transforming into an amorphous state. Therefore, not all active agents can be successfully made into nanoparticulate active agent formulations in view of the technologies available to date.

B. Wu

29. Wu et al. ("Physical and chemical stability of drug nanoparticles," *Advanced Drug Delivery Reviews*, electronically published in February, 2011, submitted herewith as Exhibit E) report that it remains challenging to obtain nanoparticulate active agent compositions that are physically and chemically stable because the stability is affected by many factors. *See* lines 84-105 and 855-861.

30. More specifically, Wu et al. teach that obtaining a stable nanoparticulate active agent composition is hindered by the difficulty of selecting a suitable surface stabilizer for the active agent. Moreover, according to Wu et al. the main challenges in designing nanoparticulate drug formulations are: (i) the lack of a fundamental understanding of the interaction between the surface stabilizer and the active agent nanoparticles (*see* lines 268-273); (ii) the process of selecting a surface stabilizer having an appropriate anchoring tail to the particular active agent is burdensome (*see* lines 268-273); (iii) the lack of predictability due to the lack of any correlation

between the physiochemical properties of the active agent and the success rate of obtaining a stable nanoparticulate active agent composition (*see* lines 399-402); and (iv) the lack of an efficient and high throughput screening technique to identify a suitable surface stabilizer (*see* lines 812-816).

C. Stable nanoparticulate clopidogrel bisulphate composition cannot be obtained.

31. Clopidogrel is an inhibitor of platelet aggregation. Clopidogrel bisulphate is insoluble in water at neutral pH but freely soluble in water at pH 1.0. Clopidogrel, as a methyl ester, is hydrolysed *in vivo* by esterases to an inactive carboxylic acid derivative, which represents more than 85% of the circulating drug related compounds in the plasma. Thus, only a small unknown portion of clopidogrel is available for metabolism to the active metabolite after oral administration. Therefore, it is desirable to obtain a nanoparticulate clopidogrel bisulphate composition to improve bioavailability of this drug.

32. The challenge in obtaining a stable nanoparticulate clopidogrel bisulphate composition is that during the milling process to reduce the particle size of clopidogrel, the pH of the milling mixture decreases while the dissolution of clopidogrel bisulphate increases. Accordingly, clopidogrel bisulphate undergoes auto-catalysis during milling process until it is completely dissolved at pH 1-2.

33. Different approaches were attempted to stabilize clopidogrel bisulphate during the milling process, such as milling in buffered systems and milling using the common ion effect, although none of these approaches resulted in a stable nanoparticulate clopidogrel bisulphate composition.

34. Milling of clopidogrel bisulphate was conducted in different buffered systems having a pH from 6.0 – 12.0 to prevent dissolution of clopidogrel bisulphate during milling. The results are detailed in the table below.

Table 7				
Clopidogrel Bisulphate (% w/w)	**Surface Stabilizer (% w/w)**	**Buffered System (pH)**	**pH prior to milling**	**pH post milling**
5%	HPC-SL (2%)	93% w/w citric acid/sodium phosphate dibasic solution (pH 7.0)	not determined	2.21
5%	Plasdone S-630 (2%)	93% w/w sodium phosphate monobasic/sodium phosphate dibasic solution (pH 8.0)	7.0	1.82
5%	Plasdone K29/K32 (2%)	93% w/w hydrochloric acid-Tris (hydroxymethyl) amino methane solution (pH 8.9)	2.0	Not milled due to dissolution of active agent prior to milling
5%	HPC-SL (2%)	93% w/w citric acid/sodium phosphate dibasic solution (pH 7.0)	6.0	2.17
5%	HPC-SL (2%)	93% w/w buffered solution (pH 12.0)	3.0	Not milled due to dissolution of active agent prior to milling
5%	Pharmacoat 603 (2%)	93% w/w simulated intestinal fluid (pH 12.0)	Not determined	2.0

35. Due to the dissolution of clopidogrel bisulphate in the buffered systems either prior to or post milling, despite the variable conditions attempted, it was found that it was impossible to obtain stable nanoparticulate clopidogrel bisulphate compositions.

36. Milling of clopidogrel bisulphate was conducted under the condition of saturating the system with common ion, bisulfate ion, in a saturated sodium bisulphate solution to control the equilibrium solubility of the clopidogrel during milling. The results are detailed in the table below.

Table 8		
Clopidogrel Bisulphate (% w/w)	**Surface Stabilizer (% w/w)**	**Observations**
5%	None	Microscopy showed the presence of drug particles in small quantities. The majority of material observed was in a flocculated state.
5%	Pharmacoat 603 (HPMC) (1%)	After subsequent addition of stabilizer, flocculation appeared to be reduced. An increased proportion of the drug appeared in the harvested aliquot suggesting increased milling of the drug. Brownian motion was not apparent.
5%	HPC-SL (2%)	Some milled drug particles were apparent although in very small quantities. Particles did not exhibit Brownian motion. Flocculation was readily apparent throughout the aliquot of sample observed under microscope.
5%	Tween 80 (2%)	Unmilled drug particles apparent. Aliquot harvested for microscopy was extremely dilute suggesting that very little drug was milled.
5%	Pharmacoat 603 (HPMC) (2%)	Nanoparticles were observed in very small quantities. Particle did exhibit a small degree of Brownian motion.
5%	Pharmacoat 603 (HPMC) (2%), DOSS (0.05%)	Very small quantities of milled drug particles were observed. Although particles were somewhat milled, they did not appear to be in the nanoparticulate size range. No Brownian motion was observed
5%	Plasdone S-630 (2%)	Harvesting was not possible as the drug did not appear to mill. No microscopy was therefore performed. A large proportion of the slurry was observed on upper mill plate and agitator possibly leading to a void in the mill chamber reducing the milling efficiency.
5%	Tyloxapol (1%)	Microscopy showed the presence of a very small concentration of milled particulates which appeared to exhibit Brownian motion most likely due to the diluted nature of the slurry.

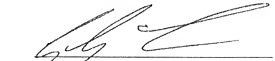
37. Despite the variable conditions attempted, it was found that it was impossible to obtain a stable nanoparticulate clopidogrel composition by controlling the equilibrium solubility of clopidogrel bisulphate via saturating the system with the common ion.

38. Accordingly, a stable nanoparticulate clopidogrel bisulphate composition could not be obtained under the various conditions tested.

CONCLUSION

39. The data described herein demonstrate unpredictability in the art, such that there is no *a priori* expectation that any given active agent could be made into a nanoparticulate composition. The data also shows that it is incorrect to assume that improved bioavailability or a reduced food effect will result merely from making the nanoparticulate form of an active agent.

40. I declare that the statements made herein of my knowledge are true and all statements on information and belief are believed to be true; and further these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing therein.



Gary Liversidge

8/4/11

Date

EXHIBIT A

US007217431B2

(12) United States Patent
Holm et al.

(10) Patent No.: US 7,217,431 B2
(45) Date of Patent: May 15, 2007

(54) **CONTROLLED AGGLOMERATION**

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(73) Assignee: **LifeCycle Pharma A/S**, Horsholm (DK)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **10/482,558**

(22) PCT Filed: **Jul. 5, 2002**

(86) PCT No.: **PCT/DK02/00472**

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(2), (4) Date: **Jul. 26, 2004**

(87) PCT Pub. No.: **WO03/004001**

PCT Pub. Date: **Jan. 16, 2003**

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Jul. 6, 2001 (DK) ..... PA 2001 01071

(51) **Int. Cl.**
A61K 9/19 (2006.01)
A61K 9/20 (2006.01)

(52) **U.S. Cl.** **424/474**; 424/78.08; 424/458; 424/459; 424/461; 424/464; 424/470; 424/497; 428/357; 428/402; 428/403; 428/407

(58) **Field of Classification Search** 428/357, 428/402, 403, 407, 424/78.08, 458, 459, 424/461, 464, 470, 474, 497
See application file for complete search history.

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Primary Examiner—Irina S. Zemel
(74) *Attorney, Agent, or Firm*—Peter F. Corless; Stephana E. Patton; Edwards Angell Palmer & Dodge LLP

(57) **ABSTRACT**

A process for the preparation of a particulate material by a controlled agglomeration method, i.e. a method that enables a controlled growth in particle size. The method is especially suitable for use in the preparation of pharmaceutical compositions containing a therapeutically and/or prophylactically active substance which has a relatively low aqueous solubility and/or which is subject to chemical decomposition. The process comprising i) spraying a first composition comprising a carrier, which has a melting point of about 5° C. or more which is present in the first composition in liquid form, on a second composition comprising a material in solid form, the second composition having a temperature of at the most a temperature corresponding to the melting point of the carrier and/or the carrier composition and ii) mixing or others means of mechanical working the second composition onto which the first composition is sprayed to obtain the particulate material.

44 Claims, 8 Drawing Sheets

Figure 1

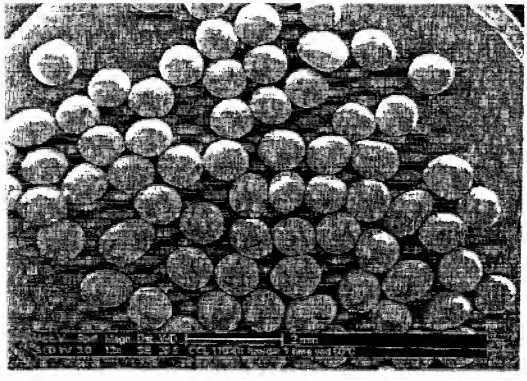

Figure 2

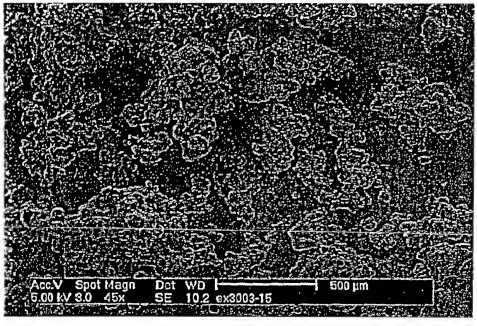

Fig. 3

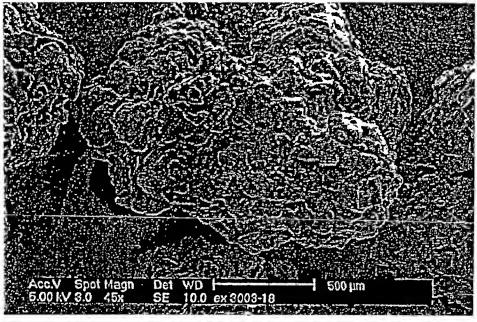

Fig. 4

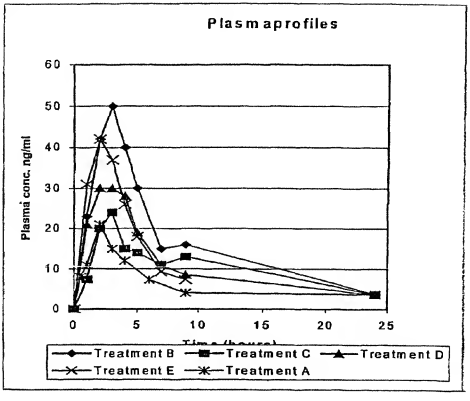
Plasma profiles
Plasma conc. ng/ml
60
50
40
30
20
10
0
0
5
10
15
20
25
Treatment B
Treatment C
Treatment D
Treatment E
Treatment A

Fig. 5

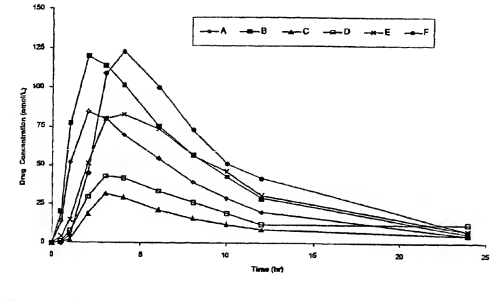
A
B
C
D
E
F
Drug Concentration (ng/mL)
Time (hr)
0
25
50
75
100
125
150
0
5
10
15
20
25

Fig. 6

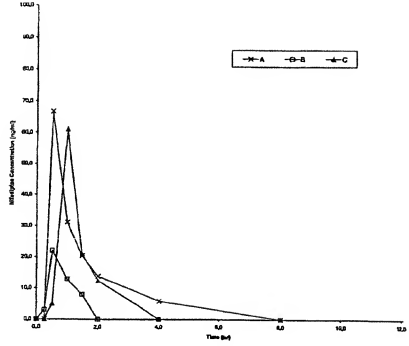

Fig. 7

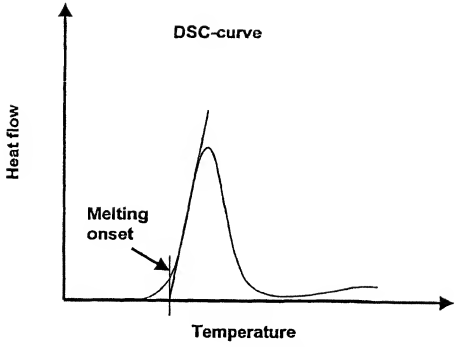

Fig 8

CONTROLLED AGGLOMERATION

FIELD OF THE INVENTION

The present invention relates to a process for the preparation of a particulate material by a controlled agglomeration method, i.e. a method that enables a controlled growth in particle size. The method is especially suitable for use in the preparation of pharmaceutical compositions containing a therapeutically and/or prophylactically active substance which has a relatively low aqueous solubility and/or which is subject to chemical decomposition. By employment of the novel process, compositions can be prepared that have improved properties with respect to release of the active substance from the composition as evidenced by in vitro dissolution test and/or with respect to improved shelf life of the compositions upon storage.

The invention also relates to a particulate material obtained by the novel process and to pharmaceutical compositions containing such particulate material. The particulate material obtained exhibits excellent flowability and compactability and possess excellent tabletting properties.

BACKGROUND OF THE INVENTION

There is a need for developing new and improved methods which enable preparation of pharmaceutical compositions for oral use that release the active substance from the composition in a suitable manner to enable an absorption of the active substance into the circulatory system.

DETAILED DISCLOSURE OF THE INVENTION

The present invention provides a method for controlled agglomeration, i.e. a controlled growth in particle size of a particulate material. Controlled agglomeration is provided using a process for the preparation of a particulate material (see below).

The invention also provides a process for the preparation of a particulate material, the process comprising

i) spraying a first composition comprising a carrier, which has a melting point of about 5° C. or more such as, e.g., about 10° C. or more, about 20° C. or more or about 25° C. or more and which is present in the first composition in liquid form, on a second composition comprising a material in solid form, the second composition having a temperature of at the most a temperature corresponding to the melting point of the carrier and/or of the carrier composition such as, e.g., a temperature of at least about 2° C., at least about 5° C. or at least about 10° C. lower than the melting point of the carrier and/or of the carrier composition, and

ii) mixing or other means of mechanical working the second composition into which the first composition is sprayed to obtain the particulate material.

The process enables incorporation in a solid material of a high load of a carrier of a type that e.g. due to its solubility properties enables a high load of therapeutically and/or prophylactically active substances with a relatively low aqueous solubility. The carrier is normally solid or semi-solid and normally it has a sticky oily or waxy character. However, the carrier may also be fluid at room temperature or even at temperature below 5° C. and in such cases it is contemplated that the process is carried out by employment of cooling of the second composition. By employment of the novel controlled agglomeration method a particulate material with a high load of carrier may be prepared and the resulting particulate material appears as a particulate powder in solid form. The particulate material obtained by the novel method has excellent properties with respect to flowability, bulk density, compactability and thus, it is suitable for use in the preparation of e.g. tablets. Although the particulate material may have a high load of a carrier of substantially sticky character the particulate material prepared has minimal, if any, adherence to tablet punches and/or dies during manufacture of tablets.

Methods for the preparation of granular products are described e.g. in EP-A-0 306 465 (Lejus Medical Aktiebolag), JP 60184378 (Takeda) and in WO 01/22941 (H. Lundbeck A/S). However, in none of these documents is described a method for the preparation of a particulate material, which method enables incorporation of a relatively high amount of a carrier as defined below and at the same time controlling the size of the particles obtained.

Carriers and Carrier Compositions

As indicated above an important step in the process for the preparation of a particulate material according to the invention is the addition of a carrier or a carrier composition. The carrier is of a type, which has a melting point of at least about 25° C. such as, e.g., at least about 30° C. at least about 35° C. or at least about 40° C. For practical reasons, the melting point may not be too high, thus, the carrier normally has a melting point of at the most about 300° C. such as, e.g., at the most about 250° C., at the most about 200° C., at the most about 150° C. or at the most about 100° C. If the melting point is higher then it becomes very difficult to ensure maintenance of a sufficient high temperature during the delivery of the carrier to the spraying equipment necessary to provide the melted carrier (or carrier composition) in the form of a spray. Furthermore, in those cases where e.g. a therapeutically and/or prophylactically active substance is included in the carrier composition, a relatively high temperature may promote e.g. oxidation or other kind of degradation of the substance.

In the present context, the melting point is determined by DSC (Differential Scanning Calorimetry). The melting point is determined as the temperature at which the linear increase of the DSC curve intersect the temperature axis (see FIG. 8 for further details).

Suitable carriers are generally substances, which are used in the manufacture of pharmaceuticals as so-called melt binders or solid solvents (in the form of solid dosage form), or as co-solvents or ingredients in pharmaceuticals for topical use.

The carrier may be hydrophilic, hydrophobic and/or they may have surface-active properties. In general hydrophilic and/or hydrophobic carriers are suitable for use in the manufacture of a pharmaceutical composition comprising a therapeutically and/or prophylactically active substance that has a relatively low aqueous solubility and/or when the release of the active substance from the pharmaceutical composition is designed to be immediate or non-modified. Hydrophobic carriers, on the other hand, are normally used in the manufacture of a modified release pharmaceutical composition. The above-given considerations are simplified to illustrate general principles, but there are many cases where other combinations of carriers and other purposes are relevant and, therefore, the examples above should not in any way limit the invention.

Examples on a suitable carrier are a hydrophilic carrier, a hydrophobic carrier, a surfactant or mixtures thereof.

Typically, a suitable hydrophilic carrier is selected from the group consisting of: polyether glycols such as, e.g.,

polyethylene glycols, polypropylene glycols, polyoxyethylenes, polyoxypropylenes, poloxamers and mixtures thereof, or it may be selected from the group consisting of: xylitol, sorbitol, potassium sodium tartrate, sucrose tribehenate, glucose, rhamnose, lactitol, behenic acid, hydroquinon monomethyl ether, sodium acetate, ethyl fumarate, myristic acid, citric acid, Gelucire 50/13, other Gelucire types such as, e.g., Gelucire 44/14 etc., Gelucire 50/10, Gelucire 62/05, Sucro-ester 7, Sucro-ester 11, Sucro-ester 15, maltose, mannitol and mixtures thereof.

Hydrophobic carrier for use in a process of the invention may be selected from the group consisting of: straight chain saturated hydrocarbons, sorbitan esters, paraffins; fats and oils such as e.g., cacao butter, beef tallow, lard, polyether glycol esters; higher fatty acid such as, e.g. stearic acid, myristic acid, palmitic acid, higher alcohols such as, e.g., cetanol, stearyl alcohol, low melting point waxes such-as, e.g., glyceryl monostearate, hydrogenated tallow, myristyl alcohol, steryl alcohol, substituted and/or unsubstituted monoglycerides, substituted and/or unsubstituted diglycerides, substituted and/or unsubstituted triglycerides, yellow beeswax, white beeswax, carnauba wax, castor wax, japan wax, acetylate monoglycerides; NVP polymers, PVP polymers, acrylic polymers, or a mixture thereof.

In an interesting embodiment, the carrier is a polyethylene glycol having an average molecular weight in a range of from about 400 to about 35,000 such as, e.g., from about 800 to about 35,000, from about 1,000 to about 35,000 such as, e.g., polyethylene glycol 1,000, polyethylene glycol 2,000, polyethylene glycol 3,000, polyethylene glycol 4,000, polyethylene glycol 5,000, polyethylene glycol 6000, polyethylene glycol 7,000, polyethylene glycol 8,000, polyethylene glycol 9,000 polyethylene glycol 10,000, polyethylene glycol 15,000, polyethylene glycol 20,000, or polyethylene glycol 35,000. In certain situations polyethylene glycol may be employed with a molecular weight from about 35,000 to about 100,000.

In another interesting embodiment, the carrier is polyethylene oxide having a molecular weight of from about 2,000 to about 7,000,000 such as, e.g. from about 2,000 to about 100,000, from about 5,000 to about 75,000, from about 10,000 to about 60,000, from about 15,000 to about 50,000, from about 20,000 to about 40,000, from about 100,000 to about 7,000,000 such as, e.g., from about 100,000 to about 1,000,000, from about 100,000 to about 600,000, from about 100,000 to about 400,000 or from about 100,000 to about 300,000.

In another embodiment, the carrier is a poloxamer such as, e.g. Poloxamer 188, Poloxamer 237, Poloxamer 338 or Poloxamer 407 or other block copolymers of ethylene oxide and propylene oxide such as the Pluronic® and/or Tetronic® series. Suitable block copolymers of the Pluronic® series include polymers having a molecular weight of about 3,000 or more such as, e.g. from about 4,000 to about 20,000 and/or a viscosity (Brookfield) from about 200 to about 4,000 cps such as, e.g., from about 250 to about 3,000 cps. Suitable examples include Pluronic® F38, P65, P68LE, P75, F77, P84, P85, F87, F88, F98, P103, P104, P105, F108, P123, F123, F127, 10R8, 17R8, 25R5, 25R8 etc. Suitable block copolymers of the Tetronic series include polymers having a molecular weight of about 8,000 or more such as, e.g., from about 9,000 to about 35,000 and/or a viscosity (Brookfield) of from about 500 to about 45,000 cps such as, e.g., from about 600 to about 40,000. The viscosities given above are determined at 60° C. for substances that are pastes at room temperature and at 77° C. for substances that are solids at room temperature.

The carrier may also be a sorbitan ester such as, e.g., sorbitan di-isostearate, sorbitan dioleate, sorbitan monolaurate, sorbitan monoisostearate, sorbitan monooleate, sorbitan monopalmitate, sorbitan monostearate, sorbitan sesquiisostearate, sorbitan sesquioleate, sorbitan sesquistearate, sorbitan tri-isostearate, sorbitan trioleate, sorbitan tristearate or mixtures thereof.

The carrier composition may of course comprise a mixture of different carriers such as, e.g., a mixture of hydrophilic and/or hydrophobic carriers.

In another interesting embodiment, the carrier is a surfactant or a substance having surface-active properties. It is contemplated that such substances are involved in the wetting of e.g. slightly soluble active substance and thus, contributes to improved solubility characteristics of the active substance.

Examples on surfactants are given in the following, in order to be suitable for use as a carrier, the criteria with respect to melting point and/or viscosity discussed herein must be fulfilled. However, the list below encompasses surfactants in general, because surfactants may also be added to the carrier composition in the form of pharmaceutically acceptable excipients.

In a process according to the invention, the carrier may be employed as such or in the form of a carrier composition. A carrier composition comprises one or more carriers optionally together with one or more other ingredients. Thus, the carrier composition may comprise a mixture of hydrophilic and/or hydrophobic carriers and/or surfactants. The carrier composition may also comprise one or more therapeutically and/or prophylactically active substances and/or one or more pharmaceutically acceptable excipients.

Suitable excipients for use in a carrier composition (and—as discussed above—for use as carriers it selves) are surfactants such as, e.g., hydrophobic and/or hydrophilic surfactants as those disclosed in WO 00/50007 in the name of Lipocine, Inc. Examples on suitable surfactants are

i) polyethoxylated fatty acids such as, e.g. fatty acid mono- or diesters of polyethylene glycol or mixtures thereof such as, e.g. mono- or diesters of polyethylene glycol with lauric acid, oleic acid, stearic acid, myristic add, ricinoleic acid, and the polyethylene glycol may be selected from PEG 4, PEG 5, PEG 6, PEG 7, PEG 8, PEG 9, PEG 10, PEG 12, PEG 15, PEG 20, PEG 25, PEG 30, PEG 32, PEG 40, PEG 45, PEG 50, PEG 55, PEG 100, PEG 200, PEG 400, PEG 600, PEG 800, PEG 1000, PEG 2000, PEG 3000, PEG 4000, PEG 5000, PEG 6000, PEG 7000, PEG 8000, PEG 9000, PEG 1000, PEG 10,000, PEG 15,000, PEG 20,000, PEG 35,000,

ii) polyethylene glycol glyceryl fatty acid esters, i.e. esters like the above-mentioned but in the form of glyceryl esters of the individual fatty acids;

iii) glycerol, propylene glycol, ethylene glycol, PEG or sorbitol esters with e.g. vegetable oils like e.g. hydrogenated castor oil, almond oil, palm kernel oil, castor oil, apricot kernel oil, olive oil, peanut oil, hydrogenated palm kernel oil and the like,

iv) polyglycerized fatty acids like e.g. polyglycerol stearate, polyglycerol oleate, polyglycerol ricinoleate, polyglycerol linolenate,

v) propylene glycol fatty acid esters such as, e.g. propylene glycol monolaurate, propylene glycol ricinoleate and the like,

vi) mono- and diglycerides like e.g. glyceryl monooleate, glyceryl diolese, glyceryl mono- and/or diolenate, glyceryl caprylate, glyceryl caprate etc.;

viii) sterol and sterol derivatives;

viii) polyethylene glycol sorbitan fatty acid esters (PEG-sorbitan fatty acid esters) such as esters of PEG with the various molecular weights indicated above, and the various Tween® series;

ix) polyethylene glycol alkyl ethers such as, e.g. PEG oleyl ether and PEG lauryl ether;

x) sugar esters like e.g. sucrose monopalmitate and sucrose monolaurate;

xi) polyethylene glycol alkyl phenols like e.g. the Triton® X or N series;

xii) polyoxyethylene-polyoxypropylene block copolymers such as, e.g., the Pluronic® series, the Synperonic® series, Emkalyx®, Lutrol®, Supronic® etc. The generic term for these polymers is "poloxamers" and relevant examples in the present context are Poloxamer 105, 108, 122, 123, 124, 181, 182, 183, 184, 185, 188, 212, 215, 217,231, 234, 235, 237, 238, 282, 284, 288, 331, 333, 334, 335, 338, 401, 402,403 and 407;

xiii) sorbitan fatty acid esters like the Span® series or Ariacel® series such as, e.g. sorbitan monolaurate, sorbitan monopalmitate, sorbitan monooleate, sorbitan monostearate etc.;

xiv) lower alcohol fatty acid esters like e.g. oleate, isopropyl myristate, isopropyl palmitate etc.;

xv) ionic surfactants including cationic, anionic and zwitterionic surfactants such as, e.g. fatty acid salts, bile salts, phospholipids, phosphoric acid esters, carboxylates, sulfates and sulfonates etc.

When a surfactant or a mixture of surfactants is present in a carrier composition the concentration of the surfactant(s) is normally in a range of from about 0.1–75% w/w such as, e.g., from about 0.1 to about 20% w/w, from about 0.1 to about 15% w/w, from about 0.5 to about 10% w/w, or alternatively, when applicable as a carrier or a part of the carrier composition from about 20 to about 75% w/w such as, e.g. from about 25 to about 70% w/w, from about 30 to about 60% w/w.

Other suitable excipients in a carrier composition may be solvents or semi-solid excipients like, e.g. propylene glycol, polyglycolised glycerides including Gelucire 44/14, complex fatty materials of plant origin including theobroma oil, carnauba wax, vegetable oils like e.g. almond oil, coconut oil, corn oil, cottonseed oil, sesame oil, soya oil, olive oil, castor oil, palm kernels oil, peanut oil, rape oil, grape seed oil etc., hydrogenated vegetable oils such as, e.g. hydrogenated peanut oil, hydrogenated palm kernels oil, hydrogenated cottonseed oil, hydrogenated soya oil, hydrogenated castor oil, hydrogenated coconut oil; natural fatty materials of animal origin including beeswax, lanolin, fatty alcohols including ceryl, stearyl, lauric, myristic, palmitic, stearic fatty alcohols; esters including glyceryl stearate, glycol stearate, ethyl oleate, isopropyl myristate; liquid interesterified semi-synthetic glycerides including Miglycol 810/812; amide or fatty acid alcolamides including stearamide ethanol, diethanolamide of fatty coconut acids etc.

Other additives in the carrier composition may be antioxidants like e.g. ascorbic acid, ascorbyl palmitate, butylated hydroxyanisole, butylated hydroxytoluene, hypophosphorous acid, monothioglycerol, potassium metabisulfite, propyl gallate, sodium formaldehylde sulfoxylate, sodium metabisulfite, sodium thiosulfate, sulfur dioxide, tocopherol, tocopherol acetate, tocopherol hemisuccinate, TPGS or other tocopherol derivatives, etc. The carrier composition may also contain e.g. stabilising agents. The concentration of an antioxidant and/or a stabilizing agent in the carrier composition is normally from about 0.1% w/w to about 5% w/w.

In those cases where a carrier composition is employed, the requirements with respect to the melting point mentioned above normally also apply to the carrier composition, especially in those cases where a minor amount of water is included in the carrier composition. However, when the carrier composition is heated the carrier composition may be in the form of two or more phases (e.g. two distinct liquid phase, or a liquid phase comprising e.g. an active substance dispersed therein). In such cases, the melting point is not a true melting point but merely a heating point where the carrier composition becomes in a liquid form, which is suitable for use in a spraying device. Often such a heating point will for practical purposes correspond to the melting point of the carrier itself.

The total concentration of carrier(s) in the carrier composition is normally in a range of from about 5 to about 100% w/w such as, e.g., from about 10 to about 99.5% w/w, from about 15 to about 99% w/w, from about 15 to about 98% w/w, from about 15 to about 97% w/w, from about 20 to about 95% w/w such as at least about 25% w/w, at least about 30% w/w, at least about 35% w/w, at least about 40% w/w, at least about 45% w/w, at least about 50% w/w, at least about 55% w/w, at least about 60% w/w, at least about 65% w/w, at least about 70% w/w, at least about 75% w/w, at least about 80% w/w, at least about 85% w/w, at least about 90% w/w, at least about 95% w/w or at least about 98% w/w.

As explained above, in a process according to the invention the carrier or the carrier composition is brought on liquid form by heating the carrier and/or the carrier composition to a temperature, which causes the carrier and/or the carrier composition to melt, and the carrier in liquid form (i.e. as a solution or a dispersion) is sprayed on the second composition.

As mentioned above, the carrier or the carrier composition in melted or liquidized form is sprayed on a second composition. Thus, the carrier or the carrier composition should have a suitable viscosity. If the viscosity is too high, the carrier or carrier composition will be too "thick" and will have a tendency of adhering to the nozzle, which may result in that the delivery through the nozzle is stopped. For the present purpose a viscosity of the carrier and/or the carrier composition is suitably if the viscosity (Brookfield DV-III) is at the most about 800 mPas at a temperature of at the most 100° C. such as, e.g., at the most 700, at the most 600, at the most 500 mPas. In those cases where the melting point of the carrier or the carrier composition is more than about 80° C., the viscosity values mentioned above are at a temperature of about 40° C. above the melting point.

In the particulate material obtained by a process according to the invention, the concentration of the carrier is from about 5 to about 95% w/w such as, e.g. from about 5 to about 90% w/w, from about 5 to about 85% w/w, from about 5 to about 80% w/w, from about 10 to about 75% w/w, from about 15 to about 75% w/w, from about 20 to about 75% w/w, from about 25% to about 75% w/w, from about 30% to about 75% w/w, from about 35% to about 75% w/w, from about 25% to about 70% w/w, from about 30% to about 70% w/w, from about 35% to about 70% w/w, from about 40% to about 70% w/w, from about 45% to about 65% w/w or from about 45% to about 60% w/w.

In those cases where the second composition comprises a pharmaceutically acceptable excipient that has a relatively high particle density it is preferred that the concentration of the carrier in the particulate material obtained by a process

of the invention is from about 5 to about 95% v/v such as, e.g., from about 5 to about 90% v/v, from about 5 to about 85% v/v, from about 5 to about 80% v/v, from about 10 to about 75% v/v from about 15 to about 75% v/v, from about 20 to abut 75% v/v, from about 25% to abut 75% v/v, from about 30% to about 75% v/v, from about 35% to about 75% v/v, from about 25% to about 70% v/v, from about 30% to about 70% v/v, from about 35% to abu 70% v/v, from about 40% to about 70% v/v, from about 45% to about 65% v/v or from about 45% to about 60% v/v.

In the following is given a calculation example:

Recalculation from % w/w to % v/v (of total composition):

Particle density of lactose: 1.56 g/cm^3

Particle density of calcium hydrogen phosphate anhydrous: 2.89 g/cm^3

Particle density of PEG 6000: 1.17 g/cm^3

For lactose: w/w ratio of 50% PEG 6000/(lactose+PEG 6000) equals a % v/v of 56% For calcium hydrogen phosphate anhydrous: w/w ratio of 50% PEG 6000/(calcium hydrogen phosphate anhydrous+PEG 6000) equals a % v/v of 71%.

In many cases it is suitable to dissolve or disperse a therapeutically and/or prophylactically active substance in the carrier or in the carrier composition. Suitable therapeutically and/or prophylactically active substances are discussed below.

In a process according to the invention it is not necessary to employ water or an aqueous medium e.g. together with a binder in order to build up agglomerates of a suitable size. The agglomeration suitably takes place under water-free or substantially water-free conditions. Thus, the process is also very useful when active substances or other ingredients are employed which are susceptible to water (e.g. degradation under aqueous conditions). However, if desired, water or an aqueous medium may of course be incorporated in the carrier composition. Although the carrier composition normally is essentially non-aqueous, water may be present to a certain extent and then the concentration of water in the carrier composition is the most about 20% w/w water such as at the most about 15% w/w, at the most abut 10% w/w, at the most about 5% w/w or at the most about 2.5% w/w.

Therapeutically and/or Prophylactically Active Substances

In a preferred embodiment of the invention the particulate material obtained by a process according to the invention comprises a therapeutically and/or prophylactically active substance. The particulate matter may also or alternatively comprise a cosmetically active substance (i.e. a substance that is employed in cosmetic compositions). In a process according to the invention the active substance may be included in the carrier composition and/or in the second composition.

In the present context a therapeutically and/or prophylactically active substance includes any biologically and/or physiologically active substance that has a function on an animal such as, e.g. a mammal like a human. The term includes drug substances, hormones, genes or gene sequences, antigen-comprising material, proteins, peptides, nutrients like e.g. vitamins, minerals, lipids and carbohydrates and mixtures thereof. Thus, the term includes substances that have utility in the treatment and/or preventing of diseases or disorders affecting animals or humans, or in the regulation of any animal or human physiological condition. The term also includes any biologically active substance which, when administered in an effective amount, has an effect on living cells or organisms.

Many active substances have and it is expected that many of the future drug substances will have undesired properties especially with respect to water solubility and to oral bioavailability. Therefore, a novel technology, which enables especially therapeutically and/or prophylactically active substances to be delivered to the body in a relatively easy manner and at the same time enables the desired therapeutic and/or prophylactic response, is highly needed.

By employment of a process according to the present invention it is contemplated that this object can be achieved for many such substances, especially in view of the promising results the inventors have obtained from a study in Beagle dogs. Accordingly, the present inventors have found very promising results with respect to bioavailability when a process according to the invention is employed for the preparation of particulate material containing an active substance with a very low aqueous solubility. Thus, a process according to the invention is especially suitable for use for the preparation of particulate material comprising an active substance that has an aqueous solubility at 25° C. and pH of 7.4 of at the most about 3 mg/ml such as, e.g., at the most about 2 mg/ml, at the most about 1 mg/ml, at the most about 750 μg/ml, at the most about 500 μg/ml, at the most about 250 μg/ml, at the most about 100 μg/ml, at the most about 50 μg/ml, at the most about 25 μg/ml, at the most about 20 μg/ml or at the most about 10 μg/ml. In specific embodiments the solubility of the active substance may be much lower such as, e.g., at the most about 1 μg/ml, at the most about 100 μg/ml, at the most about 75 μg/ml such as about 50 μg/ml.

As mentioned above a process according to the invention may advantageously be carried out without employment of water or an aqueous medium. Thus, the process is especially suitable for use for active substances that are degraded, decomposed or otherwise influenced by water.

Examples on active substances suitable for use in a particulate material according to the invention are in principle any active substance such as, e.g. freely water soluble as well as more slightly or insoluble active substances. Thus, examples on active substances suitable for use are e.g. antibacterial substances, antihistamines and decongestants, anti-inflammatory agents, antiparasites, antivirals, local anesthetics, antifungals, amoebicidals or trichomonocidal agents, analgesics, antianxiety agents, anticloting agents, antiarthritics, antiasthmatics, antiarthritic, anticoagulants, anticonvulsants, antidepressants, antidiabetics, antiglaucoma agents, antimalarials, antimicrobials, antineoplastics, antiobesity agents, antipsychotics, antihypertensives, antitussives, auto-immune disorder agents, anti-impotence agents, anti-Parkinsonism agents, anti-Alzheimers' agents, antipyretics, anticholinergics, anti-ulcer agents, anorexic, beta-blockers, beta-2 agonists, beta agonists, blood glucose-lowering agents, bronchodilators, agents with effect on the central nervous system, cardiovascular agents, cognitive enhancers, contraceptives, cholesterol-reducing agents, cytostatics, diuretics, germicidals, H-2 blockers, hormonal agents, hypnotic agents, inotropics, muscle relaxants, muscle contractants, physic energizers, sedatives, sympathomimetics, vasodilators, vasoconstrictors, tranquilizers, electrolyte supplements, vitamins, counterirritants, stimulants, anti-hormones, drug antagonists, lipid-regulating agents, uricosurics, cardiac glycosides, expectorants, purgatives, contrast materials, radiopharmaceuticals, imaging agents, peptides, enzymes, growth factors, etc.

Specific examples include e.g.

Anti-inflammatory drugs like e.g. ibuprofen, indometacin, naproxen, nabolphine;

Anti-Parkinsonism agents like e.g. bromocriptine, biperidin, benzhexol, benztropine etc.

Antidepressants like e.g. imipramine, nortriptyline, pritiptyline, etc.

Antibiotics like e.g. cliadamycin, erythomycin, fusidic acid, gentamicin, mupirocine, amfomycin, neomycin, metronidazol, sulphamethizole, bacitracin, framycetin, polymyxin B, actinomycin etc.,

Antifungal agents like e.g. miconazol, ketoconazole, clotrimazole, amphotericin B, nystatin, mepyramin, econazol, fluconazol, flucytocine, griseofulvin, bifonazole, amorolfine, mycostatin, itroconazole, terbenafine, terconazole, tolnaftate etc.

Antimicrobial agents like e.g. metronidazole, tetracyclines, oxytetracyclines, penicillins etc.

Antiemetics like e.g. metoclopramide, droperidol, haloperidol, promethazine etc.

Antihistamines like e.g. chlorpheniramine, terfenadine, triprolidine etc.

Antimigraine agents like e.g. dihydroergotamine, ergotamine, pizotyline etc.

Coronary, cerebral or peripheral vasodilators like e.g. nifedipine, diltiazem etc

Antianginals such as. e.g., glyceryl nitrate, isosorbide dinitrate, molsidomine, verapamil etc.

Calcium channel blockers like e.g. verapamil, nifedipine, diltiazem, nicardipine etc.

Hormonal agents like e.g. estradiol, estron. estriol, polyestradiol, polyestriol, dienestrol, diethylstilbestrol, progestogrone, dihydroprogesterone, cyprosterone, danazol, testosterone etc.

Contraceptive agents like e.g. ethinyl estradiol, lynestrenol, etynodiol, norethisterone, mestranol, norgestrel, levonorgestrel, desodestrel, medroxyprogesterone etc.

Antithrombotic agents like e.g. heparin, warfarin etc.

Diuretics like e.g. hydrochlorothizide, flunarizine, minoxidil etc.

Antihypertensive agents like e.g. propanolol, metoprolol, clonidine, pindolol etc.

Corticosteroids like e.g. beclomethasone, betamethasone, betamethasone-17-valerate, betamethasone-dipropionate, clobetasol, clobetasol-17-butyrate, clobetasol-propionate, desonide, desoxymethasone, dexamethasone, diflucortolone, flumethasone, flumetasone-pivalte, fluocinolone acetonide, fluocinonide, hydrocortisone, hydrocortisone-17-butyrate, hydrocortisonebutieprate, methylprednisolone, triamcinolone acetonide, hacinonide, fluprednide acetate, alklometasone-dipropionate, fluocortolone, fluticason-propionte, mometasone-furate, desoxymethasone, diflurason-diacetate, halquinol, eliochinol, chlorchinaldol, fluocinolone-acetonide etc.

Dermatological agents like e.g. nitrofurantoin, dithranol, clioquinol, hydroxyquinoline, isotretionin, methoxalen, methotrexate, tretionin, trioxalen, salicylic add, penicillamine etc.

Steroids like e.g. estradiol, progesterone, norethindrone, levonorgestrel, ethynodiol, levonorgestrol, norgestimate, gestanin, desogestrel, 3-keton-desogesterel, demegestone, promethoestrol, testosterone, spironolactone and esters thereof etc.

Nitro compounds like e.g. amyl nitrates, nitroglycerine and isosorbide nitrate etc

Opioids like e.g. morphine, buprenorphine, oxymorphone, hydromorphone, codeine, tramadol etc.

Prostaglandins such as. e.g., a member of the PGA, PGB, PGE or PGF series such as, e.g. minoprostol, dinoprostun, carboprost, eneprostil etc.

Peptides like e.g. growth hormone releasing factors, growth factors (e.g. epidermal growth factor (EGF), nerve growth factor (NGF), TGF, PDGF, insulin growth factor (IGF), fibroblast growth factor (aFGF, bFGF etc.), somatostatin, calcetonin, insulin, vasopressin, interferons, IL-2 etc., urokinase, serratiopeptidase, superoxide dismutase, thyrotropin releasing hormone, lutenizing hormone releasing hormone (LH-RH), corticotrophin releasing hormone, growth hormone releasing hormone (GHRH), oxytodin, erythropoietin (EPO), colony stimulating factor (CSF) etc.

Interesting examples on active substances that are slightly soluble, sparingly soluble or insoluble in water are given in the following tables:

TABLE 1

Poorly-Soluble Drug Candidates

Drug Name	Therapeutic Class	Solubility in Water
Alprazolam	CNS	Insoluble
Amiodarone	Cardiovascular	Very Slightly
Amlodipine	Cardiovascular	Slightly
Artemisole	Respiratory	Insoluble
Atenolol	Cardiovascular	Slightly
Azathioprine	Anticancer	Insoluble
Azelastine	Respiratory	Insoluble
Beclomethasone	Respiratory	Insoluble
Budesonide	Respiratory	Sparingly
Bupreorphine	CNS	Slightly
Butalbital	CNS	Insoluble
Carbamazepine	CNS	Insoluble
Carbidopa	CNS	Slightly
Cefotaxime	Anti-infective	Sparingly
Cephalexin	Anti-infective	Slightly
Cholestyramine	Cardiovascular	Insoluble
Ciprofloxacin	Anti-infective	Insoluble
Cisapride	Gastrointestinal	Insoluble
Cisplatin	Anticancer	Slightly
Clarithromycin	Anti-infective	Insoluble
Clonazepam	CNS	Slightly
Clozapine	CNS	Slightly
Cyclosporin	Immunosuppressant	Practically Insoluble
Diazepam	CNS	Slightly
Diclofenac sodium	NSAID	Sparingly
Digoxin	Cardiovascular	Insoluble
Dipyridamole	Cardiovascular	Slightly
Divalproex	CNS	Slightly
Dobutamine	Cardiovascular	Sparingly
Doxazosin	Cardiovascular	Slightly
Enalapril	Cardiovascular	Sparingly
Estradiol	Hormone	Insoluble
Etodolac	NSAID	Insoluble
Etoposide	Anticancer	Very Slightly
Famotidine	Gastrointestinal	Slightly
Felodipine	Cardiovascular	Insoluble
Fentanyl citrate	CNS	Sparingly
Fexofenadine	Respiratory	Slightly
Finasteride	Genito-urinary	Insoluble
Fluconazole	Antifungal	Slightly
Flunisolide	Respiratory	Insoluble
Flurbiprofen	NSAID	Slightly
Fluvoxamine	CNS	Sparingly
Furosemide	Cardiovascular	Insoluble
Glipizide	Metabolic	Insoluble
Glyburide	Metabolic	Sparingly
Ibuprofen	NSAID	Insoluble
Isosorbide dinitrate	Cardiovascular	Sparingly
Isotretinoin	Dermatological	Insoluble
Ketanserin	Cardiovascular	Insoluble
Ketoconazole	Antifungal	Insoluble
Ketoprofen	NSAID	Slightly
Lamotrigine	CNS	Slightly

TABLE 1-continued

Poorly-Soluble Drug Candidates

Drug Name	Therapeutic Class	Solubility In Water
Lansoprazole	Gastrointestinal	Insoluble
Loperamide	Gastrointestinal	Slightly
Loratadine	Respiratory	Insoluble
Lorazepam	CNS	Insoluble
Lovastatin	Cardiovascular	Insoluble
Medroxyprogesterone	Hormone	Insoluble
Mefenamic acid	Analgesic	Slightly
Melphalanmaleate	Steroid	Insoluble
Mesoridazine	Anesthesia	Insoluble
Mometasone	Steroid	Insoluble
Nabumetone	NSAID	Insoluble
Naproxen	NSAID	Insoluble
Nicergoline	CNS	Insoluble
Nifedipine	Cardiovascular	Practically Insoluble
Norfloxacin	Antiinfective	Slightly
Omeprazole	Gastrointestinal	Slightly
Paclitaxel	Anticancer	Insoluble
Phenytoin	CNS	Insoluble
Piroxicam	NSAID	Sparingly
Quinapril	Cardiovascular	Insoluble
Ramipril	Cardiovascular	Insoluble
Risperidone	CNS	Insoluble
Saquinavir	Protease inhibitor	Practically Insoluble
Sertraline	CNS	Slightly
Simvastatin	Cardiovascular	Insoluble
Terbinafine	Antifungal	Slightly
Terfenadine	Respiratory	Slightly
Tiamulin	Steroid	Insoluble
Valproic acid	CNS	Slightly
Zolpidem	CNS	Sparingly

The amount of active substance incorporated in a particulate material (and/or in a pharmaceutical, cosmetic or food composition) may be selected according to known principles of pharmaceutical formulation. In general, the dosage of the active substance present in a particulate material according to the invention depends inter alia on the specific drug substance, the age and condition of the patient and of the disease to be treated.

A particulate material according to the invention may comprise a cosmetically active ingredient and/or a food ingredient. Specific examples include vitamins, minerals, vegetable oils, hydrogenated vegetable oils, etc.

Second Composition

As mentioned above the carrier or carrier composition is sprayed on a second composition. In order to be able to achieve a high amount of carrier in the final particulate material and in order to enable a controlled agglomeration of the particles comprised in the second composition, the present inventors have surprisingly found that in specific embodiments, the second composition should initially have a temperature which is at least about 10° C, such as, e.g., at least about 15° C., at least about 20° C., at least about 25° C., or at least about 30° C. below the melting point of the carrier or carrier composition (or, as discussed above, the heating point of the carrier composition). However, as mentioned above, a temperature difference of at least about 10° C. it is not always necessary. Thus, the second composition may have a temperature of at the most a temperature corresponding to the melting point of the 2° C., at least about 5° C. No external heating of the second composition is normally employed during the process of the invention, but in some cases it may be advantageous to employ a cooling

TABLE 2

Poorly-Soluble Drugs with Low Bioavailability

Drug Name	Indication	Solubility In Water	Bioavailability
Astemizole	Allergic Rhinitis	Insoluble	Low, moderate
Cyclandelate	Peripheral vascular disease	Insoluble	Low
Perphenazine	Psychotic disorder	Insoluble	Low
Testosterone	Androgen Replacement Therapy	Insoluble	Low
Famotidine	GERD	Slightly soluble	Low (30–50%)
Budesonide	Allergic Rhinitis	Sparingly soluble	Low (~15%)
Mesalamine	Irritable Bowel Syndrome	Slightly soluble	Low (~20%)
Clemastine fumarate	Allergic Rhinitis	Slightly soluble	Low (~39%)
Bupropion hydrochloride	Pain	Slightly soluble	Low (<30%)
Sertraline	Anxiety	Slightly soluble	Low (~44%)
Aumanofin	Arthritis	Slightly soluble	Low (15–25%)
Felodipine	Hypertension	Insoluble	Low (15%)
Enalapril maleate	Hypertension	Insoluble	Low (15–54%)
Danazol	Endometriosis	Insoluble	Low
Loratadine	Allergic Rhinitis	Insoluble	Low
Loxapine succinate	Angina	Sparingly soluble	Low (20–35%)
Trifluoperazine	Psychotic disorder	Insoluble	Low (2–3%)
Spironolactone	Hypertension, Edema	Insoluble	Low (~25%)
Bupropion	Parkinson's disease	Sparingly soluble	Low (29–33%)
Cyclosporin	Transplantation	Slightly soluble	Low (30%)
Norfloxacin	Bacterial infection	Slightly soluble	Low (30–40%)
Ganciclovir	GERD	Insoluble	Low (35–40%)
Nabumetone	Arthritis	Insoluble	Low (15%)
Dronabinol	ANTIEMETIC	Insoluble	Low (10–20%)
Lovastatin	Hypertriglicemia	Insoluble	Low (~5%)
Simvastatin	Hypertriglicemia	Insoluble	Low (~5%)

via the inlet air. However, the temperature of the second composition may increase to a minor extent due to the working of the composition. However, the temperature must (or will) not be higher than at the most the melting point of the carrier or carrier composition such as, e.g. at the most about 5° C. such as at the most about 10° C., at the most about 15° C., or at the most about 20° C. below the melting point of the carrier or the carrier composition. Accordingly, a process of the invention can be carried out without any heating of the second composition, i.e. it can be carried out at ambient or room temperature (i.e. normally in a range of from about 20° C. to about 25° C.).

In contrast thereto, known melt granulation methods involve external heating of the material that is to be granulated (or agglomerated) together with a melt binder.

The second composition comprises pharmaceutically and/or cosmetically acceptable excipients and, furthermore, a therapeutically and/or prophylactically active substance may be present in the second composition.

In the present context the terms "pharmaceutically acceptable excipient" and "cosmetically acceptable excipient" are intended to denote any material, which is inert in the sense that it substantially does not have any therapeutic and/or prophylactic effect per se. Such an excipient may be added with the purpose of making it possible to obtain a pharmaceutical and/or cosmetic composition, which has acceptable technical properties.

Examples on suitable excipients for use in a second composition include fillers, diluents, disintegrants, binders, lubricants etc. or mixture thereof. As the particulate material obtained by a process according to the invention may be used for different purposes, the choice of excipients is normally made taken such different uses into considerations. Other pharmaceutically acceptable excipients for use in a second composition (and/or in the carrier composition) are e.g. acidifying agents, alkalizing agents, preservatives, antioxidants, buffering agents, chelating agents, coloring agents, complexing agents, emulsifying and/or solubilizing agents, flavors and perfumes, humectants, sweetening agents, wetting agents etc.

Examples on suitable fillers, diluents and/or binders include lactose (e.g. spray-dried lactose, α-lactose, β-lactose, Tablettose®, various grades of Pharmatose®, Microtose® or Fast-Flo®), microcrystalline cellulose (various grades of Avicel®, Elcema®, Vivocel®, Ming Tai® or Solka-Floc®), hydroxypropylcellulose, L-hydroxypropylcellulose (low substituted), hydroxypropyl methylcellulose (HPMC) (e.g. Methocel E, F and K, Metolose SH of Shin-Etsu, Ltd, such as, e.g. the 4,000 cps grades of Methocel E and Metolose 60 SH, the 4,000 cps grades of Methocel F and Metolose 65 SH, the 4,000, 15,000 and 100,000 cps grades of Methocel K, and the 4,000, 15,000, 39,000 and 100,000 grades of Metolose 90 SH), methylcellulose polymers (such as, e.g., Methocel A, Methocel A4C, Methocel A15C, Methocel A4M), hydroxyethylcellulose, sodium carboxymethylcellulose, carboxymethylene, carboxymethylhydroxyethylcellulose and other cellulose derivatives, sucrose, agarose, sorbitol, mannitol, dextrins, maltodextrins, starches or modified starches (including potato starch, maize starch and rice starch), calcium phosphate (e.g. basic calcium phosphate, calcium hydrogen phosphate, dicalcium phosphate hydrate), calcium sulfate, calcium carbonate, sodium alginate, collagen etc.

Specific examples of diluents are e.g. calcium carbonate, dibasic calcium phosphate, tribasic calcium phosphate, calcium sulfate, microcrystalline cellulose, powdered cellulose, dextrans, dextrin, dextrose, fructose, kaolin, lactose, mannitol, sorbitol, starch, pregelatinized starch, sucrose, sugar etc.

Specific examples of disintegrants are e.g. alginic acid or alginates, microcrystalline cellulose, hydroxypropyl cellulose and other cellulose derivatives, croscarmellose sodium, crospavidone, polacrilin potassium, sodium starch glycolate, starch, pregelatinized starch, carboxymethyl starch (e.g. Primogel® and Explotab®) etc.

Specific examples of binders are e.g. acacia, alginic acid, agar, calcium carrageenan, sodium carboxymethylcellulose, microcrystalline cellulose, dextrin, ethylcellulose, gelatin, liquid glucose, guar gum, hydroxypropyl methylcellulose, methylcellulose, pectin, PEG, povidone, pregelatinized starch etc.

Glidants and lubricants may also be included in the second composition. Examples include stearic acid, magnesium stearate, calcium stearate or other metallic stearate, talc, waxes and glycerides, light mineral oil, PEG, glyceryl behenate, colloidal silica, hydrogenated vegetable oils, corn starch, sodium stearyl fumarate, polyethylene glycols, alkyl sulfates, sodium benzoate, sodium acetate etc.

Other excipients which may be included in the second composition (and/or in the carrier composition) are e.g. colouring agents, taste-masking agents, pH-adjusting agents, solubilizing agents, stabilising agents, wetting agents, surface active agents, antioxidants, agents for modified release etc.

In certain cases it may be advantageously to incorporate a magnesium aluminometasilicate in the particulate material. It may be a part of the second composition or it may be added subsequently in order to facilitate a further processing of the particulate material (e.g. to prepare solid dosage forms like capsules or tablet). Magnesium aluminometasilicate is sold under the name Neusilin and is obtainable from Fuji Chemical Industries. Neusilin is normally used in order to improve filling capacity and compression property of powders and granules when added. Neusilin is also believed to reduce weight variation and to improve hardness and disintegration of tablets. Finally, Neusilin has an adsorption capability, which makes it suitable for use when processing waxy materials like oil extracts and waxes into pharmaceutical composition. Especially Neusilin UFL2 and US2 are said to be suitable for such a use.

Thus, in one aspect the invention relates to a process, wherein the second composition comprises magnesium aluminosilicate and/or magnesium aluminometasilicate such as, e.g. Neusilin S1, Neusilin FH2, Neusilin US2, Neusilin UFL2 or the like. Other suitable substances are contemplated to be bentonite, kaolin, magnesium trisilicate, montmorilonite and/or saponite. In a still further embodiment, the second composition comprises magnesium aluminosilicate and/or magnesium aluminometasilicate such as, e.g. Neusilin, and the particulate material obtained has an content of carrier of at least about 30% v/v such as, e.g. at least about 40% v/v, at Yeast about 50% v/v, at least about 60% v/v, at least about 70% v/v, at least about 75% v/v, at least about 80% v/v, at least about 85% v/v or at least about 90% v/v.

Besides the known use of Neusilin, the present inventors have found that specific qualities of magnesium aluminometasilicate (Neusilin) have excellent properties as glidants or anti-adhesive most likely due to the porous structure of Neusilin. Thus, Neusilin may advantageously be added in order to reduce any adherence of the particulate material to the manufacturing equipment in particular to the tabletting machine. in the examples herein is given a com-

parison of the anti-adhesive properties of Neusilin compared with known lubricants and Neusilin seems to be a very promising and novel candidate as a lubricant.

Details on Controlled Agglomeration

A process according to the invention may be carried out in a high or low shear mixer or in a fluid bed. Important characteristics are that the carrier or the carrier composition is sprayed on the second composition, which is loaded into the mixer or the fluid bed. Normally, the carrier or the carrier composition is heated to a temperature above the melting point of the carrier and/or the carrier composition and the second composition has not been subject to any heating and has normally ambient temperature. The difference in temperature between the carrier and the second composition makes the carrier solidify rapidly which in turn leads to a controlled growth of the particle size. Thus, the inventors have found that by employing such conditions it is possible to control the agglomeration process so that the growth in particle size is controlled.

In the present context, the term "controlled agglomeration" is intended to mean that the increase in mean geometric diameter of a material is a linear or approximated linear function of the carrier concentration in the carrier composition (see FIG. 1). Controlled agglomeration is also present if a d_{gw} of < or ~500 μm is obtained when a carrier composition containing 20% carrier has been added to a second composition.

The possibility of controlling the agglomeration makes it possible to obtain a particulate material that has a very high load of carrier(s) much higher than described when conventional methods like e.g. melt granulation is employed. As discussed above, a high load of carrier has shown to be of importance especially when particulate material is prepared containing a slightly water-soluble, sparingly water soluble or insoluble active substances. FIG. **2** is a theoretically calculated curve showing the relationship between obtainable dose and drug solubility in a carrier composition at different carrier concentrations in the particulate material assuming a total composition weight of 500 mg. It is seen that the dose can be increased by a factor of about 3.5 by increasing the concentration of carrier from 20% to 70%. By conventional melt granulation, i.e. a process by which heating of a melt binder and excipients is performed, normally a load of at the most about 15% w/w of the melt binder is obtained (calculated on the final composition). Another granulation method, which makes use of the same temperature of the binder and the material to be granulated, is a conventional granulation process, which is performed either by a wet or a dry granulation process.

A SEM micrograph in FIG. **3** shows a particulate material prepared by a process according to the present invention. PEG 6000 is used as a carrier and lactose is used as the second composition. The figure shows that the primary particles of lactose are agglomerated by immersion in the droplets of PEG 6000 or by coalescence between larger agglomerates. The agglomerates are partly coated with PEG 6000. The probability of agglomerate growth by coalescence is reduced by rapidly solidifying PEG due to the product temperature being kept at a minimum of 10° C. below the melting point of PEG.

In contrast thereto, uncontrolled agglomeration is shown in a SEM micrograph in FIG. **4**. The particulate material is prepared according to Example 2 herein (uncontrolled agglomeration) using PEG 6000 as carrier and lactose as excipients. The figure shows that the particulate material has larger agglomerates with surplus of liquefied PEG at the surface of the agglomerates increasing the probability of agglomerate growth by coalescence at elevated product temperature.

A process according to the invention may be carried out in a fluid bed. In such cases the second composition is normally kept in a fluidized state by incoming air at ambient temperature. The carrier or carrier composition is sprayed on the fluidized second composition and in order to keep the carrier or carrier composition on a liquid form and/or to avoid any clotting of the spraying device, the spraying device is kept at a suitable temperature above the melting point of the carrier or carrier composition. Normally, the spraying is performed through a spraying device equipped with temperature controlling means.

The particulate material obtained by a process of the invention has a geometre weight mean diameter d_{gw} of ≥10 μm such as, e.g. 220 μm, from about 20 to about 2000, from about 30 to about 2000, from about 50 to about 2000, from about 60 to about 2000, from about 75 to about 2000 such as, e.g. from about 100 to about 1500 μm, from about 100 to about 1000 μm or from about 100 to about 700 μm. In specific embodiments the geometric weight mean diameter d_{gw} is at the most about 400 μm or at the most 300 μm such as, e.g., from about 50 to about 400 μm such as, e.g., from about 50 to about 350μm, from about 50 to about 300 μm, from about 50 to about 250 μm or from about 100 to about 300 μm.

Particulate Material—Characteristics

Many characteristics of the particulate material obtained by a process according to the invention have already been discussed. In summary, a particulate material has good tabletting properties including good flowability and compactability. It has no or minimal adherence to the tabletting equipment either in itself or after addition of the normal amount of lubricants. It is an excellent alternative for incorporation of active substances with very low water solubility and/or with a very low bioavailability, or active substances, which are subject to degradation in the presence of water (the process may be carried out without any water).

Thus, a particulate material of the invention is excellent for a further processing into e.g. tablets. In contrast to capsules, tablets are normally easier and cheaper to produce and tablets are often preferred by the patient. Furthermore, a tablet formulation is relatively easy to adjust to specific requirements, e.g. with respect to release of the active substance, size etc.

The particulate material may also be coated (see Examples) with a film coating, an enteric coating, a modified release coating, a protective coating, an anti-adhesive coating etc.

Suitable coating materials are e.g. methylcellulose, hydroxypropylmethylcellulose, hydroxypropylcellulose, acrylic polymers, ethylcellulose, cellulose acetate phthalate, polyvinyl acetate phthalate, hydroxypropyl methylcellulose phthalate, polyvinylalcobol, sodium carboxymethylcellulose, cellulose acetate, cellulose acetate phthalate, gelatin, methacrylic acid copolymer, polyethylene glycol, shellac, sucrose, titanium dioxide, carnauba wax, microcrystalline wax, zein.

Plasticizers and other ingredients may be added in the coating material. The same or different active substance may also be added in the coating material.

Pharmaceutical Compositions

The particulate material obtained by a process according to the invention may be used as such or it may be further processed to the manufacture of a pharmaceutical and/or a

cosmetic composition by addition of one or more suitable pharmaceutically and/or cosmetically acceptable excipients. Furthermore, the particulate material obtained may be provided with a coating to obtain coated particles, granules or pellets. Suitable coatings may be employed in order to obtain composition for immediate or modified release of the active substance and the coating employed is normally selected from the group consisting of film-coatings (for immediate or modified release) and enteric coatings or other kinds of modified release coatings, protective coatings or anti-adhesive coatings

The particulate material obtained by a process of the invention is especially suitable for further processing into tablets. The material possesses suitable properties for tabletting purposes, cf. below, but in some cases it may be suitable to add further therapeutically and/or prophylactically active substances and/or excipients to the particulate material before the manufacture of tablets. For examples, by using a mixture of i) an active substance contained in modified release coated granules or granules in the form of modified release matrices and ii) an active substance in freely accessible form, a suitable release pattern can be designed in order to obtain a relatively fast release of an active substance followed by a modified (i.e. often prolonged) release of the same or a different active substance.

As appears from the above, a particulate material obtained by a process of the invention is suitable for use in the manufacture of tablets obtained by direct compression. Furthermore, the particulate material may in itself be employed as a binding agent for use in dry granulation processes.

A particulate material obtained by a process according to the invention may be employed in any kind of pharmaceutical compositions in which the use of a solid particulate material is applicable. Thus, relevant pharmaceutical compositions are e.g. solid, semi-solid, fluid or liquid composition or compositions in the form of a spray. The particulate material may also be incorporated in a suitable drug delivery device such as, e.g. a transdermal plaster, a device for vaginal use or an implant.

Solid compositions include powders, and compositions in dosage unit form such as, e.g. tablets, capsules, sachets, plasters, powders for injection etc.

Semi-solid compositions include compositions like ointments, creams, lotions, suppositories, vagitories, gels, hydrogels, soaps, etc.

Fluid or liquid compositions include solutions, dispersions such as. e.g., emulsions, suspension, mixtures, syrups, etc.

Accordingly, the invention also relates to any pharmaceutical composition comprising a particulate material obtainable by a process of the invention.

Other Aspects of the Invention

The invention also relates to a pharmaceutical particulate material obtained by mixing a first and a second composition as defined herein and heating to a temperature that is below the melting point of a carrier contained in the first composition. The heating may be applied while mixing or in a separate step. The particulate material generally has a geometric weight mean diameter d_{gw} of ≧10 um such as, e.g. ≧20 μm, from about 20 to about 2000, from about 30 to about 2000, from about 50 to about 2000, from about 60 to about 2000, from about 75 to about 2000 such as, e.g. from about 100 to about 1500 μm, from about 100 to about 1000 μm or from about 100 to about 700 μm, or at the most about 400 μm or at the most 300 μm such as. e.g., from about 50 to about 400 μm such as, e.g., from about 50 to about 350 μm, from about 50 to about 300 μm, from about 50 to about 250 μm or from about 100 to about 300 μm. In such a material the concentration of the carrier typically is at least about 40% v/v.

Such a particulate material is especially suitable for use in the preparation of solid dosage form such as tablets, capsules, sachets and the like. It may have sufficient properties with respect to flowability and/or anti-adhesion so that addition of e.g. a lubricant can be omitted when preparing a solid dosage form, especially if it comprises magnesium aluminosilicate and/or magnesium aluminametasilicate.

In a further aspect, the invention relates to the use of magnesium aluminosilicate and/or magnesium aluminometasilicate as a lubricant.

All details described herein for the main aspect of the invention apply *mutatis mutandi* to any other aspect of the invention.

LEGENDS TO FIGURES

FIG. **1** shows the correlation between amount of PEG 6000 sprayed onto lactose 125 mesh and mean granule size (geometric weight mean diameter) for a product temperature of 40–45° C. and 50–60 ° C., respectively. The dashed line indicates uncontrolled agglomeration at a PEG concentration of approx. 25% at a product temperature of 50–60° C. The products are unscreened.

FIG. **2** shows the relationship between obtainable dose and drug solubility in a carrier at different concentrations of carrier assuming a formulation unit weight of 500 mg.

FIG. **3** is a SEM micrograph of PEG sprayed onto lactose 125 mesh; the PEG concentration is 48% w/w. Magnification:45.

FIG. **4** is a SEM micrograph of PEG sprayed onto lactose 125 mesh; the PEG concentration is 25% w/w. Magnification:45.

FIG. **5** shows results from Example 4.

FIG. **6** shows mean serum concentrations vs. time profiles after p.o. administration of the model drug substance from Example 5 (30 mg) in six different formulations to Beagle dogs. Treatment A: 0.5% HPC (aq.), Treatment B: 5% Captisol® (aq.), Treatment C: Model drug substance from Example 5/SLS (2:1), Treatment D: Model drug substance from Example 5/SLS (1:1), Treatment E: Tween 80, Kollidon VA64. corn starch and lactose, Treatment F: Aksoft® 3103.

FIG. **7** shows the plasma concentration versus time curves for formulation A, B, C described in Example 6 after oral administration to dogs.

FIG. **8** illustrates determination of a melting point by a DSC curve.

The invention is further illustrated in the following examples.

Methods

Determination of Weight Variation

The tablets prepared in the Examples herein were subject to a test for weight variation performed in accordance with Ph. Eur.

Determination of Average Tablet Hardness

The tablets prepared in the Examples herein were subject to at test for tablet hardness employing Schleuniger Model 6D apparatus and performed in accordance with the general instructions for the apparatus.

Determination of Disintegration Time

The time for a tablet to disintegrate, i.e. to decompose into particles or agglomerates, was determined in accordance with Ph. Eur.

Determination of Geometric Weight Mean Diameter d_{gw}

The geometric weight mean diameter was determined by employment of a method of laser diffraction dispersing the particulate material obtained or the starting material in air. The measurements were performed at 1 bar dispersive pressure in Sympatec Helos equipment, which records the distribution of the equivalent spherical diameter. This distribution is fitted to a log normal volume-size distribution.

When used herein, "geometric weight mean diameter" means the mean diameter of the log normal volume-size distribution.

Determination of Aqueous Solubility

The aqueous solubility at 25° C. in distilled or purified water was determined by suspending a well-defined and excessive amount of the substance under investigation in a well-defined amount of distilled or purified water. The dispersion is stirred and samples are withdrawn after suitable time periods. The samples are filtered and the filtrate analysed to obtain the concentration of the substance in the sample. The concentration of the substance in the sample is then calculated according to methods well known for a person skilled in the art. The solubility is reached when the concentrations of the substance in two consecutive samples are considered identical.

Determination of Dissolution Rate

The dissolution rate was determined by employment of USP paddle dissolution method at 37° C.

Materials

All materials employed were of pharmaceutical grade.
Calcium hydrogen phosphate (Di-cafos A): Budenheim
Croscarmellose Sodium Ac-Di-Sol: FMC
Magnesium stearate: Magnesia GmbH
Polyethylene glycol: Hoechst
Lactose: DMV
Other materials employed appear from the following examples.

EXAMPLES

Example 1

Preparation of Tablets Containing a Particulate Material According to the Invention

The example illustrates the preparation of a particulate material comprising a relatively large amount of a carrier. The particulate material obtained exhibits good flowability, good compactability and possesses excellent tabletting properties. Thus, the particulate material allow the preparation of e.g. tablets and in spite of the relatively large load of carrier the tablets display minimal, if any, adherence (sticking) to tablet punches and/or dies during compression. Furthermore, the tablets obtained have acceptable properties with respect to disintegration, weight variation and hardness.

Starting Materials

Lactose monohydrate (DMV) 125 mesh
Calcium hydrogen phosphate anhydrous (Di-Ca-Fos P)
Polyethylene glycol 6000 (PEG 6000) having a melting point of about 60° C.

Equipment

Fluid bed Strea-1 (from Aeromatic-Fielder) mounted with a special developed top-spray binary nozzle having an opening of 0.8 mm.

Granular Compositions

Composition 1.1	
Lactose	600 g
PEG 6000	420 g (sprayed on lactose)

The composition has a carrier concentration of 45.6% w/w.

Composition 1.2	
Calcium hydrogen phosphate anhydrous	900 g
PEG 6000	210 g (sprayed on calcium hydrogen phosphate)

The composition has a carrier concentration of 29.6% w/w.

Process Conditions—Description

Lactose (or for composition 1.2 calcium hydrogen phosphate anhydrous) was fluidised at appropriate inlet airflow. The inlet air was not heated. PEG 6000 was melted using an electrically heated pressure tank. The temperature was kept at a temperature at about 85° C., i.e. above the melting point of PEG 6000. The melt was pumped from the tank to the nozzle through a heated tube, in the tube, the temperature was kept at 80° C. The pressure in the tank determined the flow rate of the melt. The nozzle was heated to keep the droplets in a liquefied stage by means of heating the atomizer air delivered through the top-spray nozzle.

Settings

Inlet airflow: 30–50 m^3 per hour
Inlet air temperature: Ambient temperature (20–25° C.)
Tank temperature: 85° C.
Tank pressure: 1.5 Bar corresponding to a flow rate of 14–15 g/min
Tube temperature: 80° C.
Atomising air temperature: 100° C.
Process time: 28 min
Product temperature at equilibrium: 40° C. (after 15 minutes)

Product Characteristics

The products (composition 1.1 and 1.2) appear as free flowing granular products with a mean granule size of approx. 300–500 μm.

Tabletting

Compositions

Tablet formulation 1 (without disintegrant)	
Granular product	99% w/w
Magnesium stearate	1% w/w

The tablet formulation has a carrier concentration of 45.2% w/w.

Tablet formulation II (with disintegrant)	
Granular product	95% w/w
AcDiSol (croscarmellose sodium)	4% w/w (disintegrant)
Magnesium stearate	1% w/w

The tablet formulation has a carrier concentration of 28% w/w.

Tablet Properties

Tablet formulation I based on composition I.1, i.e. with lactose

Tablet punch: Compound cup, 10 mm in diameter
Tablet machine: Single punch machine Korsch EK0
Tablet weight: 250 mg
Weight variation, RSD <1%
Average tablet hardness: 96 N
Average disintegration time: 10 min
Tablet appearance: White glossy tablets

Tablet formulation I based on composition I.2, i.e. with dicalcium phosphate

Tablet punch: Compound cup, 10 mm in diameter
Tablet machine: Single punch machine Korsch EK0
Tablet weight: 450 mg
Weight variation, RSD <1%
Average tablet hardness: 121 N
Average disintegration time: 17 min
Tablet appearance: White glossy tablets

Tablet formulation II based on composition I.1, i.e. with lactose

Tablet punch: Compound cup, 10 mm in diameter
Tablet machine: Single punch machine Korsch EK0
Tablet weight: 250 mg
Weight variation, RSD <1%
Average tablet hardness: 112 N
Average disintegration time: 8 min
Tablet appearance: White glossy tablets

Thus, addition of a disintegrant results in a decrease in the average disintegration time without any other changes of importance.

Tablet formulation II based on composition I.2, i.e. with calcium hydrogen phosphate

Tablet punch: Compound cup, 10 mm in diameter
Tablet machine: Single punch machine Korsch EK0
Tablet weight: 450 mg
Weight variation, RSD <1%
Average tablet hardness: 118 N
Average disintegration time: 9 min
Tablet appearance: White glossy tablets

When calcium dihydrogen phosphate anhydrous is employed a more pronounced decrease in disintegration time is observed compared with that of lactose. The average tablet hardness is maintained at an excellent level.

Example 2

Controlled Agglomeration—Proof of Concept

Method

Controlled agglomeration is obtained by keeping the product temperature at minimum 10° C. below melting point of the carrier reducing the probability of agglomeration due to coalescence. Controlled agglomeration is characterised by gradual increase in mean granule size (geometric weight mean diameter d_{gw}) as function of applied amount of carrier. In contrast, uncontrolled agglomeration shows rapidly increasing granule size. As a proof of concept the granule growth pattern are compared corresponding to the following conditions:

Inlet fluidising air temperature of ambient temperature: 20–25° C.

Inlet fluidising air temperature of 85° C. leading to a temperature of the product of about 50–60° C.

Starting Materials

Lactose monohydrate 125 mesh

Polyethylene glycol 6000

Equipment

Fluid bed Strea-1 mounted with a top-spray binary nozzle.

Granular Compositions

Lactose 400 g
PEG 6000 Increased steps se in separate experiments (from 0% to about 60% w/w in the final composition)

Process Conditions

The conditions were the same as described in Example 1.

Settings (Controlled Agglomeration)

Inlet airflow: 30–50 m^3 per hour
Inlet air temperature: Ambient temperature (20–25° C.)
Tank temperature: 90° C.
Tank pressure: 1.5 Bar corresponding to a flow rate of 14–15 g/min
Tube temperature: 85° C.
Atomizer air temperature: 100° C.
Product temperature at equilibrium: 40° C.

Settings (Uncontrolled Agglomeration)

Inlet airflow: 30–50 m^3 per hour
Inlet air temperature: 85° C.
Tank temperature: 90° C.
Tank pressure: 1.5 Bar corresponding to a flow rate of 14–15 g/min
Tube temperature: 85° C.
Atomizer air temperature: 100° C.
Product temperature at equilibrium: 55–65° C.

Product Characteristics

Increasing amounts of PEG were sprayed onto the fluidised lactose particles and the particle size distribution of the products was analysed by method of laser diffraction, dispersing the agglomerates in air. The correlation between mean granule size (geometric weight mean diameter d_{gw}) and applied amount of carrier demonstrates the difference between controlled and uncontrolled agglomeration as shown in FIG. 1 and Table 1. Table 1 includes the geometric standard deviation s_g related to the wideness of the size distribution.

TABLE 1

Particle size characteristics of granulate products produced by granulation by melt spraying in fluid bed at heated and unheated inlet air conditions at different applied amount of PEG 6000 concentrations.

	Product temperature 40-45° C. Inlet air temperature: Ambient			Product temperature 50-60° C. Inlet air temperature: 55° C.		
PEG % w/w	D_{gw}, μm	S_g	PEG % w/w	D_{gw}, μm	S_g
1	93	2.17	11	95	2.17
17	151	2.04	13	143	1.98
20	261	2.09	15	513	1.48
38	328	2.06	28	960	1.43
48	332	1.95			
60	450	1.8			

D_{gw} Geometric weight mean diameter.
S_g Geometric standard deviation.

Example 3

Improving tabletting Characteristics of Paracetamol Applying the controlled Agglomeration Technique

Paracetamol has been chosen as model substance representing a substance with poor compression characteristics. By incorporation of PEG 6000 by melt spraying, i.e. spraying melted PEG 6000 on paracetamol, a granular product of paracetamol is obtained with excellent flowability and tablet compression characteristics. In order to obtain tablets with satisfactory disintegration time Avicel PH 200 and Kollidon CL (super-disintegrant) has been added to the product

Starting Materials

Polyethyleneglycol 6000 (Hoechst)

Paracetamol (Unikem)

Equipment

Fluid bed Strea-1 (Aeromatic-Fielder)

Process Conditions

300 g PEG 6000 was melted by heating to 90° C. in a pressure tank. The melted carrier was pumped through a heated tube (85° C.) to the binary nozzle in the fluid bed at a tank pressure of 1.5 Bar. The atomizing air was heated to 140° C. The inlet air temperature of the fluid bed was 25° C.

241 g of PEG was sprayed on 250 fluidized paracetamol at a flow rate of 17 g/min. The total yield was 491 g granulate with a composition corresponding to 49.1% w/w PEG 6000 and 50.9% w/w paracetamol. The maximum product temperature was 36° C. at the end of the process.

Product Characteristics

The median particle size on volume basis is 85 μm for paracetamol was increased to 295 μm during the controlled agglomeration process. The median particle size was determined by laser diffraction (Helos) dispersing the particles in air.

Tablet composition	
Paracetamol	44%
PEG 6000	41%
Avicel PH200	10%
Kollidon CL	4%
Magnesium stearate	1%

Paracetamol and PEG 6000 are employed in the form of the granular product obtained as described above.

Avicel PH is blended with the granular product for 2 minutes in Turbula mixer and after adding magnesium stearate for further 0.5 minutes. Avicel PH200 (microcrystalline cellulose) is supplied by FMC, Kollidon CL by BASF and magnesium stearate by Magnesia GmbH.

Tabletting and Tablet Characteristics

The tabletting was performed on a single punch tabletting machine Korsch EK0.

Tablet shape 8 mm doomed shape

Weight: 200 mg

Strength 87 mg

Mean tablet hardness (n=10) determined on a Schleuninger Model 6D apparatus was 77 N

Friability was 0.2% determined at a Roche friabilitator

Mean disintegration time was 11 minutes (Ph.Eur)

Weight variation (n=20) corresponded to RSD of 0.6%

In conclusion, the tablets obtained from the granulate prepared by the controlled agglomeration method of the invention were very satisfactory and only a relatively small concentration of tabletting excipients was needed in order to ensure a suitable tabletting process. Furthermore, the example demonstrates that it is possible to obtain a granulate that has a relatively high concentration of carrier (about 50% w/w) and at the same time has a suitable particle size for further processing.

Example 4

In Vivo Bioavailability in Dogs After Administration of Tablets Containing a Particulate Material Obtained by the Controlled Agglomeration Method of the Present Invention—Proof of Concept

The present example illustrates that a composition containing a particulate material obtained according to the present invention leads to improved bioavailability after oral administration to dogs compared with compositions made by techniques that are generally accepted as useful when an increase in bioavailability is desired. In the present example compositions in the form of a nanosuspension and a microemulsion are used for comparison.

The model drug substance employed illustrates a drug substance that has a very low aqueous solubility of less than 50 ng/ml independent on pH. The molecular weight of the model drug substance is about 600 and it has a lipophilicity i.e. a log P (octanol/water) of 5.0.

Proof of concept is based on a comparison of bioavailability of different oral formulations and an I.V. injection of the drug substance in dogs (n=4). Data on the I.V. is not included in this example.

Treatment Compositions and Treatment Schedule

Treatment A (comparison treatment): nanosuspension containing 2% w/w of the model drug substance. NanoCrystal™ colloidal suspension of the model drug substance stabilised with hydroxy propyl cellulose (HPC-SL). Supplier: Elan pharmaceutical technologies, USA. EPT Ref.

NB: GOI-5747-170. The nanosuspension contains 2% of the model drug substance and 1% HPC-SL (w/w). A treatment consisted in oral administration of 36.3 mg as a sigle dose (approximately 1.8 ml).

Treatment B (according to the invention): tablets containing a particulate material obtained according to the method of the present invention. The tablets contain about 1% w/w of the model drug substance. The preparation of the composition used in Treatment B is described below. A treatment consisted in oral administration of 6 tablets as a single dose corresponding to approx. 37.5 mg.

Treatment C (according to the invention): tablets containing a particulate material obtained according to the method of the present invention. The tablets contain about 5% w/w of the model drug substance. The preparation of the composition used in Treatment C is described below. A treatment consisted in oral administration of 2 tablets as a single dose corresponding to approx. 42.4 mg.

Treatment D (comparison treatment): capsules containing a microemulsion of the model drug substance. Soft gelatine capsules containing 7.3 mg of the model drug substance in a vehicle consisting of 40% w/w Softigen 767, 15% w/w triethylcitrate and 45% w/w polysorbate 80 (0.05% BHA was added by weight as antioxidant). A treatment consisted of a single dose of 5 capsules, equivalent to 36.5 mg of the model drug substance.

Treatment E (comparison treatment): capsules containing a microemulsion of the model drug substance. Soft gelatine capsules containing 12.43 mg of the model drug substance in a vehicle consising of 40% w/w Softigen 767, 15% w/w triethylcitrate and 45% w/w polysorbate 80 (0.05% BHA was added by weight as antioxidant). A treatment consisted of a single dose of 3 capsules, equivalent to 37.2 mg of the model drug substance.

Preparation of a Pharmaceutical Composition According to the Invention used in Treatment B (5 mg Model Drug Substance)

Preparation of a Particulate Material—Melt-spraying Process

Starting Materials

Polyethyleneglycol 6000 (Hoechst)
Poloxamer 188 (BASF)
Model drug substance
Avicel PH 101 (FMC)

Equipment

Fluid bed Strea-1 (Aeromatic-Fielder)

Process Conditions

198.0 g PEG 6000 and 85.0 g Poloxamer 188 (70:30 w/w) were melted by heating to 75° C. in a pressure tank. 6.21 g model drug substance was dissolved in the melted carriers. The melt was pumped through a heated tube (80° C.) to the binary nozzle in the fluid bed at a tank pressure of 1.8 Bar. The atomizing air was heated to 140° C. The inlet air temperature of the fluid bed was 22° C.

289 g of melt was sprayed on 300 g fluidized Avicel PH 101 at a flow rate of 10 g/min. The total yield was 589 g granulate. The maximum product temperature was 36° C. at the end of the process.

Product Characteristic

Granular, free flowing product with a particle size under 0.7 mm.

Tablet Composition (w/w)

Tablets were obtained by compression of a powder blend containing the granulate obtained as described above with magnesium stearate.

Model drug substance	1.04%
PEG 6000	33.29%
Poloxamer 188	14.29%
Avicel PH101	50.43%
Magnesium stearate	1.00%

Magnesium stearate was blended with the granulate for 0.5 minutes in a Turbula-mixer.

Tabletting and Tablet Characteristics

The tabletting was performed on a single punch tabletting machine Korsch EK0

Tablet shape 11.5 mm doomed shape
Weight: 515 mg
Strength 5 mg

Mean tablet hardness (n=10) determined on a Schleuninger Model 6D apparatus was 105 N

Mean disintegration time was 21.5 minutes (Ph.Eur)

Weight variation (n=20) corresponded to RSD of 0.9%

Preparation of a Pharmaceutical Composition According to the Invention used in Treatment C (20 mg Model Drug Substance)

Preparation of a Particulate Material—Melt-spraying Process

Starting Materials

Polyethyleneglycol 6000 (Hoechst)
Poloxamer 188 (BASF)
Model drug substance
Avicel PH 101 (FMC)

Equipment

Fluid bed Strea-1 (Aeromatic-Fielder)

Process Conditions

121.9 g PEG 6000 and 52.3 g Poloxamer 188 (70:30 w/w) were melted by heating to 75° C. in a pressure tank. 20.96 g model drug substance was dissolved in the melted carriers. The melt was pumped through a heated tube (80° C.) to the binary nozzle in the fluid bed at a tank pressure of 1.8 Bar. The atomizing air was heated to 140° C. The inlet air temperature of the fluid bed was 22° C.

195 g of melt was sprayed on 200 g fluidized Avicel PH 101 at a flow rate of 11.4 g/min. The total yield was 395 g granulate. The maximum product temperature was 37° C. at the end of the process.

Product Characteristic

Granular, free flowing product with a particle size under 0.7 mm.

Tablet Composition (w/w)

Tablets were obtained by compression of a powder blend containing the granulate obtained as described above with magnesium stearate.

Model drug substance	7.29%
PEG 6000	to 54%
Poloxamer 188	3.14%
Water PHEur	to 54%
Magnesium stearate	1.57%

Magnesium stearate was blended with the granulate for 0.5 minutes in a Turbula-mixer.

Tabletting and Tablet Characteristics

The tabletting was performed on a single punch tabletting machine Korsch EKO

Tablet shape 11.5 mm doomed shape

Weight: 409 mg

Strength 20 mg

Mean tablet hardness (n=10) determined on Schleuninger Model 6D apparatus was 41 N

Mean disintegration time was 5.5 minutes (Ph Eur)

Weight variation (n=20) corresponded to RSD of 1.3%

Study Design and Results

The study design was a cross-over study, which comprised all four dogs in one group, in each of totally six weeks the dogs were dosed orally on the first day of the week following by 6 days of recovery. The first week the dogs were assigned to treatment A, second week to treatment B etc.

Summary of pharmacokinetic parameters. Beagle dogs after single oral dosing of the model drug substance (±SD, n=4).

Treatment	A	B	C	D	E
t_{max} (h)	2.3 ± 0.5	2.8 ± 0.5	4.3 ± 3.2	2.8 ± 1.3	2.0 ± 0.0
C_{max} (ng/ml)	19 ± 8	32 ± 15	29 ± 17	35 ± 13	42 ± 6
$AUC_{0-\infty}$ [a] (ng*h/ml)	295 ± 108	489 ± 187	260 ± 184	318 ± 248	318 ± 65
$F^{[b]}$ (%)	5.8 ± 1.9	11 ± 4	5.4 ± 2.7	7.8 ± 3.8	7.6 ± 2.9

[a] Calculated as
[a] $AUC_{0-\infty} = C_{max} * t_{1/2,\nu} / \ln 2$;
[b] $AUC_{0-\infty,iv/po} \cdot D_{iv/po} / (AUC_{0-\infty,iv} \cdot D_{po})$

From the results given above it and in FIG. 5 is seen that treatment B leads to improved bioavailability compared with all other treatments employed. It is particularly interesting to note that compositions containing the model drug substance in dissolved form (treatment D and E) do not lead to a better bioavailability than treatment B and there is no significant difference in the t_{max} values obtained, i.e. the onset of the therapeutic effect is the same even if a solid composition is used. Treatment C leads to a lower bioavailability than treatment B, which may be explained by the fact that the ration between the amount of drug substance in the carrier is higher in treatment C than in treatment B (higher dose in treatment C than in treatment B).

Example 5

In Vivo Bioavailability in Dogs After Administration of Tablets Containing a Particulate Material Obtained by the Controlled Agglomeration Method of the Present Invention—Proof of Concept II

The present example illustrates that a composition containing a particulate material obtained according to the present invention leads to improved bioavailability after oral administration to dogs compared with compositions made by techniques that are generally accepted as useful when an increase in bioavailability is desired. In the present example compositions in the form of a nanosuspension and a cyclodextrin solution are used for comparison.

The model drug substance employed illustrates a drug substance that has a very low aqueous solubility of about 50 pg/ml in phosphate buffer pH 7.4. The model drug substance in this example has a pK_a of 8, a molecular weight of about 450 and a lipophilicity i.e. a log P (octanol/buffer pH 7.4) of 6.0. The model drug substance is employed in the form of a hydrochloride salt. The aqueous solubility of the salt is also very low.

The results presented below are based on absorption study in dogs comparing 6 different formulations.

Formulation A (nanosuspension)

Formulation B: Cyclodextrin solution (Captisol)

Formulation C: Mixture of SLS and the model drug substance (0.5:1)

Formulation D: Mixture of SLS and the model drug substance (1:1)

Formulation E: Granulate with 10% Tween 80

Formulation F: (granulate in capsule) prepared by a method according to the present invention by melt spraying and using Akosoft XP 3103.

A summary of the pharmacokinetic report on the study is given below.

Test formulation A was prepared by suspending nanonised model drug substance particles in a veliicle of 0.5% HPC (HPC) (Klucel® MF EP, Hercules Inc.) and purified water.

A similar suspension was included in an initial study where it resulted in a mean relative bioavailability of only 0.64 when compared to a 5% Captisol® solution. However, it was suspected that the initial suspension used was not optimal as the particle size distribution was above the micrometer range. Subsequently, the micronisation process has been optimised, and test formulation A was prepared from a model drug substance batch, which contained particles in the nanometer range.

Reference formulation B was prepared by dissolving the model drug substance in an aqueous vehicle of 5% β-cyclodextrin sulfobutyl ether, sodium salt (Captisol®, CyDex Inc).

Test formulation C was prepared by dissolving sodium lauryl sulphate (SLS) in water and adding the solution to the model drug substance drop by drop (model drug substance/SLS w/w-ratio 2:1). The dried mixture and lactose were filled in capsules.

Test formulation D was prepared by dissolving SLS in water and adding the solution to the model drug substance drop by drop (model drug substance/SLS w/w-ratio 1:1). The dried mixture and lactose were filled in capsules.

Test formulation E was prepared by melt granulation of the model drug substance, 10% Tween 80, 2% Kollidon VA64, corn starch and lactose. The granulate was filled in capsules.

Test formulation F was prepared by a method of the invention by melt-spraying the model drug substance, Akosoft 3103 and lactose. The granulate obtained was filled in capsules. Akosoft 3103 is a mixture of Akofine III (C_8 C_{10} monoglycerides), Akosoft 36 (hydrogenated cocoglyceride) and Akofine NF (hydrogenated cottonseed oil) from Karlshamns AB. All are saturated fats or oils, i.e. no double-bonds, PEG-chains or free acid groups exist in the excipients.

In the following the preparation of test formulation F is described in further details.

Test Formulation F

Preparation of a Particulate Material—Melt-spraying Process

Starting Materials

Akosoft XP 3103 (Karlshamn)
Model drug substance
Lactose 350 M (DMV)

Equipment

Fluid bed Strea-1 (Aeromatic-Fielder)

Process Conditions

153 g Akosoft XP 3103 was melted by heating to 70° C. in a pressure tank. The melt was pumped through a heated tube (80° C.) to the binary nozzle in the fluid bed at a tank pressure of 0.3 Bar. The atomizing air was heated to 140° C. The inlet air temperature of the fluid bed was 22° C.

114 g of melt was sprayed on fluidized material consisting of 256.5 g lactose 350 M and 43.5 g model drug substance at a flow rate of 30 g/min. The total yield was 414 g granulate. The maximum product temperature was 32° C. at the end of the process.

Product Characteristic

Granular product with a particle size under 0.7 mm.

The product was filled into capsules (500 mg corresponding to 30 mg base)

Study Design and Dosing

The study was conducted in a cross-over design. After a five days pre-dose period the test formulations were administered in intervals of three or four days. Test formulations were administered in the order B, A, C, D, E and F.

On days of dosing each dog was dosed in the morning with 30 mg of the model drug substance (with regard to the base) irrespective of bodyweight. The dose level chosen was based on previous studies with the model drug substance in Beagle dogs.

Pharmacokinetic Results

Mean serum concentrations vs. time are presented in FIG. 6. Standard deviations are omitted in the figure for clarity. The data is shown in the Table below

The concentration of the model drug substance in the serum sample taken from dog F1131 at 24 hours is high compared to the concentrations observed at previous time points. Re-analysis confirmed the result and the late serum concentration increase might therefore be due to delayed absorption of the test compound.

Pharmacokinetic parameters for the model drug substance estimated by standard non-compartmental analysis are given in the following Table.

For the reference solution a mean t_{max} of 2.5 hours was observed. The other treatments resulted in mean t_{max} values of 2.3 hours (HPC—formulation A), 3.0 hours (model drug substance/SLS 2:1—formulation C), 3.8 hours (Akosoft 3103—formulation F), 4.8 hours (Tween 80/Kollidon VA64—formulation E) and 8.3 hours (model drug substance/SLS 1:1—formulation D). The latter mean t_{max} value is high due to the extreme contribution from dog F1131 (see above). If this data point is omitted a mean t_{max} of 3.0 hours is observed.

With a mean maximum serum concentration at 123 nmol·L^{-1} the Akosoft 3103 formulation (formulation F) gave a value almost similar to the reference solution at 124 nmol·L^{-1}. At the other extreme the treatments with SLS (formulations C and D) resulted in mean C_{max} values of 31.5 nmol·L^{-1} (model drug substance/SLS 2:1) and 50.3 nmol·L^{-1} (model drug substance/SLS 1:1). Again the mean value would be smaller if the 24 hours data point for formulation D was omitted. Administration of the HPC- and Tween 80/Kollidon VA64-formulations resulted in mean C_{max} values of 87.9 nmol·L^{-1} and 85.3 nmol·L^{-1}, respectively.

In the Table on next page are given individual and mean (n=4) pharmacokinetic parameters of the model drug substance employed in Example 5 after dosing of 30 mg to Beagle dogs. Treatment A: 0.5% HPC (aq.), Treatment B: 5% Captisol® (aq.), Treatment C: model drug substance/SLS (2:1), Treatment D: model drug substance/SLS (1:1), Treatment E: Tween 80, Kollidon VA64, corn starch and lactose, Treatment F: Akosoft® 3103.

Treatment[a]	Animal	Dose[b] (mmol/kg)	t_{max} (h)	C_{max} (nmol · L^{-1})	AUC_{0-t} (nmol · h^{-1} · L^{-1})	AUC_{0-inf} (nmol · h · L^{-1})	AUC_{0-t}/AUC_{0-inf}	$t_{1/2}$[c] (h)	CLF (L · kg^{-1} · h^{-1})	V_zF (L · kg^{-1})	$F_{rel,inf}$[b]	F_{rel}[c]
A	F1131	4381	2.0	82.3	617	657	0.1	3.9	8.67	37.6	0.58	0.57
	F1132	4440	2.0	61.3	407	418	2.7	4.3	10.6	66.2	0.52	0.52
	F1138	4595	3.0	109	1025	1067	4.0	4.9	4.31	30.6	0.95	0.94
	F1139	5016	2.0	99.0	751	780	2.7	4.7	6.40	44.0	1.16	1.14
	Mean		2.3	87.9	700	731	4.1	4.5	7.0	44.6	0.77	0.77
	CV %		21.7	23.7	37.0	36.9	34.9	9.85	37.5	34.3	33.9	33.9
B (reference)	F1131	4770	3.0	145	1163	1231	5.5	5.3	3.96	29.6	—	—
	F1132	4794	3.0	101	842	873	3.5	4.7	5.49	37.2	—	—
	F1138	4995	2.0	141	1180	1217	3.0	4.7	4.11	27.6	—	—
	F1139	5560	2.0	107	804	832	3.4	4.7	6.71	45.4	—	—
	Mean		2.5	124	997	1038	3.9	4.9	5.0	35.0	—	—
	CV %		23	18	20	21	29	9.1	27	23	—	—

-continued

Treatment[a]	Animal	Dose[b] (mg/kg)	T_{max} (h)	C_{max} (nmol/L)	AUC_{0-t} (nmol/h·L^{-1})	AUC_{0-inf} (nmol/h·L^{-1})	AUC_{0-inf}/dose	$t_{1/2}$[c] (h)	CL/F[c] (L/kg^{-1}·h^{-1})	V/F[c] (L/kg^{-1})	F_{rel}[d]
C	31431	4762	4.0	12.5	78	85	17	4.1	903.2	2967	0.98	0.97
	31432	4764	3.0	8.83	53	66	26	4.3	733.0	4144	0.98	0.96
	31433	5030	3.0	9.86	54	73	30	6.1	693.3	6098	0.98	0.94
	31439	5380	3.0	9.86	78	817	44	4.9	8.83	58.2	0.98	0.97
	Mean		3.0	31.0	241	263	179	4.9	59	152.1	0.98	0.89
	CV %		0.0	41	140	141	59.9	18.8	61.0	67.9	151	158
D	31431	4730	24	32.5	321	910	97	6.2	2.75	9.89	0.11	0.28
	31432	4926	2.0	34.1	291	329	54	4.7	4.52	19.1	0.27	0.23
	31438	4944	3.0	27.7	236	249	51	5.2	20.9	150	0.20	0.20
	31439	5437	4.0	107	913	957	46	4.9	5.90	41.1	1.16	1.14
	Mean		5.3	50.3	440	559	17.7	5.3	12.2	80.0	0.44	0.46
	CV %		127	75.3	72.1	90.0	113	12.6	54.3	52.2	111	98.3
E	31431	4826	6.0	43.2	575	752	24	10	8.42	95.4	0.60	0.68
	31432	4859	6.0	28.8	802	835	43	4.5	5.42	38.1	0.94	0.94
	31438	5030	3.0	40.2	956	1015	58	5.5	4.95	39.0	1.03	0.91
	31439	5537	4.0	117	1038	1118	54	6.2	5.90	37.4	1.35	1.33
	Mean		4.8	57.3	848	930	45	6.3	5.5	52.5	0.93	0.89
	CV %		33.3	72.7	24.8	17.9	37.1	39.7	12.8	56.5	33.7	39.6
F	31431	4762	3.0	152	1334	1414	57	5.3	3.37	25.6	1.14	1.14
	31432	4825	4.0	99.1	839	867	33	4.4	5.56	35.1	0.99	0.99
	31438	5102	4.0	88.4	881	926	48	5.0	5.51	40.1	0.74	0.73
	31439	5537	4.0	153	1210	1266	44	4.8	4.37	30.1	1.53	1.52
	Mean		3.8	123	1066	1118	46	4.9	4.7	32.7	1.10	1.10
	CV %		13.2	27.9	23.9	23.6	21.6	7.70	22.2	19.2	30.0	31.0

Individual doses used in the pharmacokinetic analysis were calculated by D×CF/M_w BW. D is the dose administered with respect to the base (ng), M_w is the molecular weight of the model drug substance (ng/nmol), BW is the body weight of the animal (kg) and CF is the correction factor determined from analysis of the test formulations.

$t_{1/2}$ was calculated from λ-values estimated from data points at 2–8 hours (I), 2–12 hours (II), 2–24 hours (III), 3–12 hours (IV), 3–24 hours (V), 4–24 hours (VI), 6 hours (VII) and 6–24 hours (VIII)

$$F_{rel,\ inf} \text{ was calculated as } \frac{F_{rel} \cdot AUC_{0-inf}^{test} \text{ Dose}^{ref}}{AUC_{0-inf}^{ref} \text{ Dose}^{test}}$$

$$F_{rel,\ t} \text{ was calculated as } \frac{F_{rel} \cdot AUC_{0-t}^{test} \text{ Dose}^{ref}}{AUC_{0-t}^{ref} \text{ Dose}^{test}}$$

Mean values for the relative bioavailability (relative to cyclodextrin solution) were almost identical irrespective of the calculation being made with respect to the serum concentration time curve to infinity (AUC_{0-inf}) or to the last measurable concentration (AUC_{0-t}). Mean values for the latter AUC parameter were 997 nmol·h·L^{-1} (reference formulation), 1066 nmol·h·L^{-1} (Akosoft 3103), 848 nmol·h·L^{-1} (Tween 80/Kollidon VA64), 700 nmol·h·L^{-1} (HPC) 440 nmol·h·L^{-1} (model drug substance/SLS 1:1) and 241 nmol·h·L^{-1} (model drug substance/SLS 2:1). The low values for the two SLS formulations are in line with the low C_{max} values observed for these formulations.

The corresponding mean relative bioavailability-values were 1.10 (Akosoft 3103), 0.89 (Tween 80/Kollidon VA64), 0.77 (HPC), 0.46 (model drug substance/SLS 1:1) and 0.29 (model drug substance/SLS 2:1).

The low relative bioavailability observed for the two SLS-formulations was not expected as a similar formulation, albeit with a model drug substance/SLS-ratio at 2:1, administered in a previous study resulted in a mean relative bioavailability of 1.20. Apparently there is a critical concentration below which the dissolution- and absorption enhancing properties of SLS are limited.

All formulations administered to animal F 1039 resulted in a relative bioavailability (based on AUC_{0-inf}) around or above unity (range 0.98–1.53). The relative bioavailability determined in this dog for the different formulations therefore contributes considerably to the mean F_{rel}. This is especially the case for the two SLS formulations where the relative bioavailability is very low for the other three dogs. When this dog was excluded mean values of 0.24 and 0.06 were found for model drug substance/SLS-ratios of 1:1 and 2:1, respectively.

The mean apparent half life determined after administration of the various treatments were 4.5 hours (HPC suspension), 4.8 hours (5% Captisol® and model drug substance/SLS 2:1), 4.9 hours (Akosoft 3103), 5.2 hours (model drug substance/SLS 1:1) and 6.4 hours (Tween 80/Kollidon VA64). Mean oral clearances (CL/F) were comparable for treatments with HPC (7.01 L·kg^{-1}·h^{-1}), 5% Captisol® (5.04 L·kg^{-1}·h^{-1}), Tween 80/Kollidon VA64 (5.54 L·kg^{-1}·h^{-1}) and Akosoft 3103 (4.70 L·kg^{-1}·h^{-1}). As a consequence of the low AUC_{0-inf} values the two treatments with SLS show relatively high CL/F values at 12 L·k^{-1}·h^{-1} (model drug substance/SLS 1:1) and 50 L·$^{-1}$·h^{-1} (model drug substance/SLS 2:1).

Mean volumes of distribution (V/F) observed were 29.6 L·k^{-1} (HPC), 32.7 L·k^{-1} (Akosoft 3103), 34.9 L·$^{-1}$ (5% Captisol®) and 52.5 L·$^{-1}$ (Tween 80/Kollidon VA64). Again the values for the two SLS formulations were relatively higher at 158 L·k^{-1} and 352 L·k^{-1}.

Pharmacokinetic parameters estimated for the reference solution were consistent with values found in a previous formulation study performed on identical animals.

As supplement to these data other formulations have been prepared including Captisol formulations: B (similar to the one in the previous study), and three formulations prepared according to the invention, formulation G, H and I. These formulations include mixtures of glycerides. Formulations G, H and I (granulate in capsule) have been manufactured by melt spraying.

Preparation of Test Formulation G, H and I According to the Invention

Preparation of a Particulate Material—Melt-spraying Process

Starting Materials

Kimel C 8–50 (Mono-diglycerid on medium chain fatty acids) (Cognis)

Viscoleo (medium chain triglycerides) (Grünau Illertissen)

Rylo MG 18 Pharma (Danisco Cultor)

Sodium lauryl sulfate (Millcheim Limited)

Ascorbyl palmitate (Merck)

Model drug substance (the same substance is used throughout Example 5)

Lactose 350 M (DMV)

Equipment

Fluid bed Strea-1 (Aeromatic-Fielder)

Compositions

Material	Formulation G g	Formulation H g	Formulation I g
Rylo MG 18	25.8	25.8	25.8
Viscoleo	21.2	21.2	21.2
Kimel	21.2	21.2	21.2
Model drug	50.2	50.2	25.1
Lactose 350 M	202.1	288.1	279.7
SLS	40.0	–	–
Ascorbyl palmitate	1.8	1.8	1.8

Process Conditions

The process conditions are similar for the formulation G, H and I. Rylo MG 18 was melted by heating to 70° C. in a pressure tank and the liquids Viscoleo and Kimol were added. The melt was pumped through a heated tube (80° C.) to the binary nose in the fluid bed at a tank pressure of 0.2 Bar. The atomizing air was heated to 140° C. The inlet air temperature of the fluid bed was 22° C.

The melt was sprayed on fluidized material consisting of the particulate materials, which include the model drug substance, lactose and ascorbyl palmitate and for formulation G; sodium lauryl sulfate. The flow rate was 20 30 g/min. The maximum product temperature was 32° C. at the end of the process

Product Characteristic

Granular product with a particle size under 0.7 mm.

The product was filled into capsules (250 mg corresponding to 30 mg base for Formulation G and H). 500 mg corresponding to 30 mg base for formulation I.

Example 6

Proof of Concept Based on Data From Development Project with Nifedipine

Nifedipine is a yellow crystalline substance, practically insoluble in water with a solubility of <56 mg/L at 25° C. It has a molecular weight of 346.3 and a melting range between 172–174° C. The calculated log P is 2.5 and the experimental measured value is 2.2. Nifedipine is rapidly and fully absorbed after oral administration of the marketed products, however an immediate release capsule only produce a bioavailability between 30 and 60%.

Proof of concept is based on a comparison of bioavailability of different oral formulations with a solution of the drug substance as reference, in dogs in a cross over design. A summary is given below including detailed information on the melt spraying process and tabletting (Treatment B and C)

Treatment A

Solution of nifedipine in PEG 400

Composition

Nifedipine	2% w/w
PEG 400	98% w/w

1 ml per capsule (corresponds to 20 mg nifedipine)

Treatment B

Plan tablet 20 mg Adalat® Bayer

Treatment C

Tablets prepared from a particulate material produced according to the present invention by melt spraying. Nifedipine is contemplated to be present in PEG/poloxamer as a solid solution.

Melt-spraying Process

Starting Materials

Polyethyleneglycol 6000 (Hoechst)

Poloxamer 188 (BASF)

Nifedipine (Sigma-Aldrich)

Lactose 200 mesh (DMV)

Equipment

Fluid bed Strea-1 (Aeromatic-Fielder)

Process Conditions

254.6 g PEG 6000 and 113.4 g Poloxamer 188 (70:30 w/w) were melted by heating to 90° C. in a pressure tank. 15.27 g drug substance was dissolved in the melted carriers. The melt was pumped through a heated tube (85° C.) to the binary nozzle in the fluid bed at a tank pressure of 1.6 Bar. The atomizing air was heated to 140° C. The inlet air temperature of the fluid bed was 22° C.

508 g of melt was sprayed on 300 g fluidized lactose at a flow rate of 17 g/min. The total yield was 608 g granulate. The maximum product temperature was 37° C. at the end of the process.

Product Characteristic

Granular, free flowing product with a particle size under 0.7 mm

Tablet composition

Nifedipine	3.94% w/w
PEG 6000	37.71% w/w
Poloxamer 188	14.45% w/w
Avicel PH101	38.86% w/w
Magnesium stearate	5.04% w/w

Magnesium stearate was blended with the granulate for 0.5 minutes in a Turbula-mixer.

Tabletting and Tablet Characteristics

The tabletting was performed on a single punch tabletting machine Korsch EK0

Tablet shape 8 mm compound shape

Weight: 200 mg

Strength 5 mg

Mean tablet hardness (n=10) determined on a Schleuniger model 6D was 97 N

Mean disintegration time was 11.3 minutes (Ph.Eur)

Weight variation (n=20) corresponded to RSD of 1.15%

Dosing 4 tablets (20 mg) in a capsule

Dosing

One dog was dosed with the 3 different formulations A, B and C with 3 days between dosing. 2 ml of blood samples were taken at pre-dose and 0.25, 0.5, 1, 1.5, 2, 4, 8 and 24 hours after administration. The analysis of nifedipine was performed on respective plasma samples.

Pharmacokinetic Results

The pharmacokinetic data are shown in the Table below

formulation	A	B	C
t_{max} (h)	0.5	0.5	1.0
C_{max} (ng/ml)	66.6	22.0	61.0
AUC_{0-inf} * (ng/ml*h)	172.2	22.2	53.1
F_{rel} ** (%)	100	12.9	30.8

Calculated as

*$AUC_{0-inf} = C_{last}/\lambda_z$,

**$AUC_{0-inf,rel} = D_{0,rel}(AUC_{0-inf,rel} * D_{0,rel})$

The bioavailability F_{rel} is calculated relative to formulation A, representing a solution of nifedipine in PEG 400. The corresponding plasma profiles are shown in FIG. 7.

Conclusion

Apparently the solid solution of nifedipine in PEG6000/Poloxamer (formulation C) results in significant higher bioavailability compared to a plain tablet formulation (Adalat).

Example 7

Neusilin as Absorption Material in Controlled Agglomeration

Background

It is established that magnesium aluminium silicate (Carrisorb, Gelsorp, Magnabite) is suitable in absorption of liquids and commonly used as a viscosity increasing, a tablet disintegrant and a tablet binding agent.

Neusilin (Fuji Chemical Industries) is a magnesium aluminometasilicate based on a polymeric reaction of sodium silicate having a siloxane structure (U.S. Pat. No. 3,959,444) in combination with a mixture or sodium aluminate and magnesium salts.

Neusilin US2 is a spray dried free flowing material with a particle size of approx. 80 μm and a specific surface area of 300 m^2/g.

Two experiments (A and B) have been performed where PEG 6000 is sprayed on fluidized Neusilin in a fluid bed Strea-1.

Experiment A is performed under conditions of controlled agglomeration keeping the temperature difference over 10° C. between the product and the melting point of PEG 6000 (59° C.).

Experiment B is performed under heating condition of the inlet air (50–70° C.) resulting in a product temperature under the 10° C. temperature difference.

Experiment A

Equipment

Fluid bed Strea-1 (Aeromatic-Fielder)

Process Conditions

1000 g PEG 6000 was melted by heating to 90° C. in a pressure tank. The melt was pumped through a heated tube (85° C.) to the binary nozzle in the fluid bed at a tank pressure of 1.5 Bar. The atomizing air was heated to 140° C. The inlet air temperature of the fluid bed was 22° C.

584 g of melt was sprayed on 150 g fluidized Neusilin US2 at a flow rate of 19 g/min. The total yield was 734 g granulate. The maximum product temperature was 45° C. at the end of the process. The concentration of PEG 6000 in the particulate material obtained was 79.6% w/w.

Product Characteristic

Granular, free flowing product with a particle size d_{gw} of 409 μm.

Tablet composition	
PEG 6000	79.6%
Neusilin	20.4%

Tabletting and Tablet Characteristics

The tabletting was performed on a single punch tabletting machine Korsch EK0. It was not necessary to add further excipients for the tabletting procedure.

Tablet shape 8 mm compound cup

Weight: 200 mg

Mean tablet hardness (n=10) determined on a Schleuniger model 6D was 48.6 N

Mean disintegration time was 22.4 minutes (Ph.Eur)

Weight variation (n=20) corresponded to RSD of 0.6%

Experiment B

Equipment

Fluid bed Strea-1 (Aeromatic-Fielder)

Process Conditions 800 g PEG 6000 was melted by heating to 90° C. in a pressure tank. The melt was pumped through a heated tube (85° C.) to the binary nozzle in the fluid bed at a tank pressure of 1.5 Bar. The atomizing air was heated to 140° C. The inlet air temperature of the fluid bed was 60° C.

505 g of melt was sprayed on 150 g fluidized Neusilin US2 at a flow rate of 19 g/min. The total yield was 655 g granulate. The maximum product temperature was 58° C. at the end of the process.

Product Characteristic

Granular, free flowing product with a particle size under 0.7 mm.

Tablet composition	
PEG 6000	77.1%
Neusilin	22.9%

Tabletting and Tablet Characteristics

Tabletting was not possible due to adhesion to the punches.

Conclusion

Neusilin US2 acts as an absorption agent for the melted carrier sprayed on the fluidized material.

Surprisingly high amount of carrier was applicable corresponding to a total amount of carrier exceeding 80% without getting uncontrolled agglomeration. In Experiment A, the temperature difference between product and melting point of the carrier exceeded 10° C. Further, direct tabletting of the product without adding lubricant was successfully performed.

Increasing the inlet temperature of the fluidized bed (Experiment B) exceeding the temperature limits for controlled agglomeration (recognized for the traditionally employed excipients) did not result in uncontrolled agglomeration as expected. This is most likely due to the high absorption capacity of Neusilin preventing free surface liquid to form bondings between the fluidized particles. However, uncontrolled agglomeration occurred at the end of the process (77.1% PEG 6000). Direct compression of the product was not possible due to adhesion to the punches indicating surface free PEG in the agglomerates, which might be due to the elevated product temperature in the agglomeration process.

To sum up, it is possible to obtain controlled agglomeration even in those cases where no or only a small temperature difference is present between the carrier and the second composition. This applies especially for substances like Neusilin and the like.

Example 8

Lubricant Effect of Neusilin in Comparison with Magnesium Stearate and Aerosil 200

A sticky granulate was produced by controlled agglomeration. PEG 1500 (melting range of from about 44 to about 48° C.) was applied on lactose 200 mesh in a fluid bed Strea-1. The composition of the product was as follows:

Lactose 200 mesh	500 g
PEG 1500	250 g

The granulate was sieved through a 0.71 mm mesh size.

A part of the granulate was blended with the different substances for 3 minutes in a Turbula mixer in order to determine any lubricating effect. Two of the substances used, namely magnesium stearate and Aerosil, are known lubricants. The substances employed were:

Neusilin ULF2 (Fuji Chemical Industries)

Magnesium stearate (Magnesia GmbH)

Aerosil 200 (colloidal silicon dioxide), (Degussa AG)

Tablets were produced on a single punch tabletting machine Korsch EK0, instrumented with force transducer on the filling device measuring the force to push off the tablet from the lower punch.

Tablet diameter 8 mm. Tablet shape: Compound cup

Tablet weight: 200 mg

The results are summarised in the Table below

Lubricant	Conc. %	Adhesion to Tablet Punches	Mean Push off force N
Neusilin	2	no	4.5
	4	no	ca. 4
Magnesium stearate	1	Adhesion	n.m.
Aerosil 200	0.5	Adhesion	n.m.
	1	Adhesion	n.m.

Conclusion

Neusilin and Aerosil provided excellent flowability to the sticky granular product, whereas magnesium stearate did not have this effect. Aerosil is normally used as lubricant in the concentrations below 0.5% and is primarily used to improve the flowability of cohesive materials.

The anti-adhesive property of Neusilin is superior to both magnesium stearate and Aerosil. Granules blended with either 2 or 4% of Neusilin was compressed without any adhesion to the punches. As shown in the Table the adhesion to the lower punch was significantly decreased when increasing the concentration of Neusilin from 2 to 4%. The push off force was not monitored (n.m.) for the other lubricants since compression of tablets was not possible due to immediately adhesion to the punches.

Thus, the results demonstrate that Neusilin is an excellent lubricant having anti-adhesive properties.

The invention claimed is:

1. A method for preparing particulate material, comprising:

i) spraying a first composition on a second composition, wherein the first composition comprises one or more therapeutically or prophylactically active substances and a carrier in liquid form, wherein the carrier has a melting point of at least about 5° C., and the second composition comprises a material in solid form at a temperature corresponding to or below the melting point of the first composition, and

ii) agglomerating the first composition with the second composition to obtain the particulate material, wherein the therapeutically or prophylactically active substance has an aqueous solubility of at most about 3 mg/ml at 25° C. and a pH of about 7.4 and wherein the particulate material obtained comprises a geometric weight mean diameter from between about 75 to about 2000 μm.

2. The method of claim 1, wherein the therapeutically active or prophylactic substance has an aqueous solubility of at most about 1 mg/ml at about 25° C. and a pH of about 7.4.

3. The method of claim 1, wherein the therapeutically or prophylactically active substance has an aqueous solubility of at most about 0.01 mg/ml at about 25° C. and a pH of about 7.4.

4. The method of claim 1, wherein the carrier has a melting point of about 10° C. or more.

5. The method of claim 1, wherein the carrier has a melting point of at least about 20° C.

6. The method of claim 1, wherein the carrier has a melting point of at least about 25° C.

7. The method of claim 1, wherein the temperature of the second composition is at least about 2° C. below the melting point temperature of the carrier or the first compositon.

8. The method of claim 1, wherein the temperature of the second composition is at least about 5° C. below the melting point temperature of the carrier or the first composition.

9. The method of claim 1, wherein the temperature of the second composition is at least about 10° C. below the melting point temperature of the carrier or the first composition.

10. The method of claim 1, which agglomeration is carried out in a high shear mixer, a low shear mixer, or a fluid bed.

11. The method of claim 1, which agglomeration is carried out in a fluid bed and wherein the first composition is sprayed on the second composition in a fluidized state.

12. The method of claim 1, wherein the spraying is performed through a spraying device equipped with temperature controlling means.

13. The method of claim 1, wherein the particulate material has a geometric weight mean diameter d_{gw} of at least about 10 micrometer.

14. The method of claim 1, wherein the particulate material has a geometric weight mean diameter d_{gw} of between about 20 micrometer and about 2000 micrometer.

15. The method of claim 1, wherein the concentration of the carrier in the particles is from about 5 to about 95% v/v.

16. The method of claim 1, wherein the first composition is liquefied by heating the carrier or the first composition to a temperature, which causes the carrier or the carrier composition to melt.

17. The method of claim 16, wherein the liquefied carrier or carrier composition has a viscosity (Brookfield DV-II) of at most about 800 mPas at a temperature of at most about 100° C.

18. The method of claim 1, wherein the first composition is essentially non-aqueous and contains at most about 20% w/w water.

19. The method of claim 1, wherein the carrier has a melting point of at most about 300° C.

20. The method of claim 1, wherein the carrier is a hydrophilic carrier, a hydrophobic carrier, a surfactant or a mixture thereof.

21. The method of claim 20, wherein the carrier is selected from one or more of polyether glycols; polyoxyethylenes, polyoxypropyleneas, poloxamers and mixtures thereof.

22. The method of claim 20, wherein the carrier is selected from one or more of polyethylene glycol and polypropylene glycol.

23. The method of claim 20, wherein the carrier is selected from one or more of xylitol, sorbitol, potassium sodium tartrate, sucrose tribehenate, glucose, rhamnose, lactitol, behenic acid, hydroquinon monomethyl ether, sodium acetate, ethyl fumarate, myristic acid, citric acid, polyglycolized glycerides Gelucire 50/13, Gelucire 44/14, Gelucire 50/10, Gelucire 62/05; Sucro-ester 7, Sucro-ester 11, Sucro-ester 15, maltose, mannitol and mixtures thereof.

24. The method of claim 20, wherein the carrier is selected from one or more of straight chain saturated hydrocarbons, sorbitan esters paraffins; fats and oils, cacao butter, beef tallow, lard, polyether glycol esters; higher fatty acids, stearic acid, myristic acid, palmitic acid, higher alcohols, cetanol, stearyl alcohol, low melting point waxes, glyceryl monostearate, hydrogenated tallow, myristyl alcohol, stearyl alcohol, substituted and/or unsubstituted monoglycerides, substituted and/or unsubstituted diglycerides, substituted and/or unsubstituted triglycerides, yellow beeswax, white beeswax, camauba wax, castor wax, japan wax, acetylate monoglycerides, NVP polymers, PVP polymers, and acrylic polymers.

25. The method of claim 1, wherein the carrier is polyethylene glycol having an average molecular weight from between about 400 to about 35,000.

26. The method of claim 1, wherein the carrier is selected from the group consisting of polyethylene glycol 1,000, polyethylene glycol 2,000, polyethylene glycol 3,000, polyethylene glycol 4,000, polyethylene glycol 5,000, polyethylene glycol 6,000, polyethylene glycol 7,000, polyethylene glycol 8,000, polyethylene glycol 9,000 polyethylene glycol 10,000, polyethylene glycol 15,000, polyethylene glycol 20,000, and polyethylene glycol 35,000.

27. The method of claim 1, wherein the carrier is polyethylene oxide having a molecular weight of from between about 2,000 to about 7,000,000.

28. The method of claim 1, wherein the carrier is a poloxamer.

29. The method of claim 1, wherein the carrier is selected from the group, consisting of Poloxamer 188, Poloxamer 237, Poloxamer 338 and Poloxamer 407.

30. The method of claim 1, wherein the carrier is selected from the group consisting of sorbitan esters, sorbitan, diisostearate, sorbitan dioleate, sorbitan monolaurate, sorbitan monoisostearate, sorbitan monooleate, sorbitan monopalmitate, sorbitan monostearate, sorbitan sesqui-isostearate, sorbitan sesquioleate, sorbitan sesquistearate, sorbitan tri-isostearate, sorbitan trioleate, sorbitan tristearate and mixtures thereof.

31. The method of claim 1, wherein the first composition comprises a mixture of one or more of hydrophilic and hydrophobic carriers.

32. The method of claim 1, wherein the second composition comprises one or more therapeutically active or prophylactic substances.

33. The method of claim 1, wherein the first composition further comprises one or more pharmaceutically acceptable excipients.

34. The method of claim 33, wherein the pharmaceutically acceptable excipient is selected from the group consisting of fillers, binders, disintegrants, glidants, coloring agents, taste-masking agents, pH-adjusting agents, solubilizing agents, stabilizing agents, wetting agents, surface active agents and antioxidants.

35. The method of claim 1, wherein the second composition comprises one or more pharmaceutically acceptable excipients.

36. The method of claim 35, wherein the pharmaceutically acceptable excipient is one or more of fillers, binders, disintegrants, glidants, coloring agents, taste-masking agents, pH-adjusting agents, solubilizing agents, stabilizing agents, wetting agents, surface active agents, and antioxidants.

37. The method of claim 1, wherein the first or the second composition comprises a cosmetically active substance, a beneficial substance, a food substance, or a nutrient substance.

38. The method of claim 1, wherein the second composition comprises magnesium aluminosilicate or magnesium alumometasilicate and the amount of carrier in the particulate material is at least about 30% v/v.

39. The method of claim 38, wherein the amount of carrier in the particulate material is at least about 40% v/v.

40. The method of claim 38, wherein the amount of carrier in the particulate material is at least about 50% v/v.

41. The method of claim 1, wherein the particulate material is suitable for use in the preparation of pharmaceutical, cosmetic or food composition in a liquid, semi-solid or solid form.

42. The method of claim 1, wherein the particulate material is suitable for use in the preparation of tablets.

43. A method for improving the shelf-life of a pharmaceutical composition comprising an oxidation-sensitive therapeutically or prophylactically active substance, comprising subjecting the substance, before or during manufacture of the pharmaceutical composition, to the method of claim 1 by incorporating the substance in the first composition.

44. A method for preparing particulate material comprising spraying, on a second composition in a fluidized state, an amount of a first composition effective for agglomerating the second composition to obtain the particulate material, wherein the first composition comprises one or more therapeutically or prophylactically active substances having an aqueous solubility of at most about 3 mg/ml at 25° C. and a pH of about 7.4 and a carrier in liquid form; wherein the carrier has a melting point of at least about 5° C., the second composition comprises a material in solid form at a temperature corresponding to or below the melting point of the first composition, and the particulate material obtained has a geometric weight mean diameter from between about 75 to about 2000 μm.

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EXHIBIT B

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In vivo evaluation of matrix pellets containing nanocrystalline ketoprofen

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Abstract

The aim of this study was to evaluate the in-vivo behaviour of matrix pellets formulated with nanocrystalline ketoprofen after oral administration to dogs. No significant differences in AUC-values were seen between pellet formulations containing nanocrystalline or microcrystalline ketoprofen and a commercial ketoprofen formulation (reference: Rofenid® 200 Long Acting). C_{max} of the formulations containing nano- or microcrystalline ketoprofen was significantly higher compared to reference, whereas t_{max} was significantly lower. The in-vivo burst release observed for the spray dried nanocrystalline ketoprofen matrix pellets was reduced following compression of the pellets in combination with placebo wax/starch pellets. These matrix tablets sustained the ketoprofen plasma concentrations during 5.6 and 5.4 h for formulations containing nano- and microcrystalline ketoprofen, respectively. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Ketoprofen; Nanocrystals; Sustained release; Matrix; In vivo

1. Introduction

Ketoprofen [2-(3-benzoylphenyl) propionic acid] is a non-steroidal anti-inflammatory and analgesic agent used to treat acute and chronic rheumatoid arthritis and osteo-arthritis. Because of its short plasma elimination half-life (2–4 h), it is an interesting molecule to formulate as a slow release preparation (Habib and Mesue, 1995). As ketoprofen is poorly soluble in acidic conditions due to its pH dependent solubility profile and as poor solubility is generally related to a low bioavailability, this presents a major challenge during drug formulation. Micronization of drug particles has often been used to increase the bioavailability of poorly water soluble molecules, however reducing the particle size to the micron range has not always been sufficient to achieve this goal (Kondo et al., 1993). However, a further

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reduction of the particle size to the nanometer range has been successfully used to improve the bioavailability (Liversidge and Cundy, 1995; Liversidge and Conzentino, 1995; Müller et al., 1999), as an outstanding feature of nanosuspensions is the ability to increase the saturation solubility of the compound. In general, the saturation solubility is defined as being a compound-specific constant depending on the temperature and the crystalline structure of the compound. However, the saturation solubility is also a function of the particle size, the size-dependency only coming into effect for particles having a size below 1 μm (Müller et al., 2001). In a previous study (Vergote et al., 2001), matrix pellets containing spray dried nanocrystalline ketoprofen were developed, the main advantage of incorporating spray dried nanocrystalline ketoprofen into the matrix pellets being the pH independent in vitro drug release from these pellets. The present study was designed to evaluate the bioavailability in dogs after oral administration of nanocrystalline ketoprofen formulated in matrix pellets as well as in tablets (manufactured using a binary mixture of ketoprofen matrix pellets and placebo wax/starch pellets).

2. Materials and methods

2.1. Materials

Microcrystalline ketoprofen was obtained from Spectrum Quality Products (New Brunswick, NJ, USA). The NanoCrystal® colloidal ketoprofen dispersion (containing 20% w/w ketoprofen) was supplied by Elan Pharmaceutical Technologies (King of Prussia, PA, USA) and prepared using a ball milling process (Liversidge et al., 1991). Paraffinic wax (Paracera® P) was purchased from Paramelt (Heerhugowaard, The Netherlands), while both waxy maltodextrin (WMD) and drum dried corn starch (DDCS) were provided by Eridania-Beghin Say (Vilvoorde, Belgium). Sodium starch glycolate (Explotab®) was obtained from Penwest (Patterson, NY, USA). Rofenid® 200 Long Acting (coated pellets) (Rhône-Poulenc Rorer, Brussels, Belgium) was used as a reference formulation.

2.2. Spray drying

A NanoCrystal® ketoprofen dispersion and a microcrystalline ketoprofen dispersion (both containing 20% (w/w) ketoprofen and 0.15% (w/w) sodium laurylsulphate) were separately spray dried using a mini-spray dryer (Büchi 190, Flawil, Switzerland). Before spray-drying, the dispersions were diluted with water (1:2). The inlet temperature of the drying air was set at 75 °C (which is substantially lower than the melting point of ketoprofen (94 °C)), while the outlet temperature was 49 °C. The feeding rate of the dispersion was 200 ml/h. The particle size of the spray dried product was determined by photon correlation spectroscopy (Autosizer 4700, Malvern Instruments, Malvern, UK) in case of nanocrystalline ketoprofen and by laser diffraction (Mastersizer S, Malvern Instruments, Malvern, UK) in case of microcrystalline ketoprofen. Prior to particle sizing, the drug was suspended in deionized water at a concentration of 20% (w/w) and diluted to obtain the appropriate concentration range for particle size measurement. The mean particle size and polydispersity index (PI) of the nanocrystalline material was 265 nm and 0.24, respectively, and 65 μm and 0.32 for microcrystalline ketoprofen. Based on image analysis of the suspensions, no particles above 500 nm and 300 μm were detected for nanocrystalline and microcrystalline ketoprofen, respectively.

2.3. Production of pellets

The matrix pellets containing spray dried nanocrystalline ketoprofen (15% w/w) were produced in a laboratory scale high shear mixer (Mi-Pro, ProCept, Zelzate, Belgium), while the pellets containing spray dried microcrystalline ketoprofen (15% w/w) were produced in a Gral 10 (Machines Collette, Wommelgem, Belgium) high shear mixer. The matrix of both pellet formulations consisted of 35% (w/w) wax, 6.5% (w/w) drum dried corn starch and 43.5% (w/w) waxy maltodextrin. The parameters during pellet production were selected based on the method described by Vergote et al. (2001). The pellet fraction between 800 and 1000 μm was isolated by

sieving (Retsch VE 1000, Retsch, Haan, Germany) and used for further analysis.

2.4. Production of tablets

The tablets used in the in-vivo study were composed of a binary mixture of ketoprofen pellets (loaded with 15% (w/w) nano- or microcrystalline drug) and placebo beads in a ratio of 50:50% (w/w). The placebo pellets (800–1200 μm), made by melt pelletisation, consisted of 50% (w/w) paraffinic wax, 33.3% (w/w) drum dried corn starch and 16.7% (w/w) Explotab®. The pellet mixture was manually filled into the die (diameter: 13 mm) and tabletted at a compression force of 10 kN using a single punch tablet press (Korsch EKO, Germany). All tablet formulations contained 100 mg ketoprofen.

2.5. In vitro dissolution testing

An in-vitro dissolution test of the pellets and tablets was performed in phtalate buffer pH 4.6 (without enzymes, USP XXIII) according to the method described by Vergote et al. (2001).

2.6. In vivo evaluation

The pellet formulations were administered to six mixed bred dogs (three males and three females), while the in-vivo behaviour of the tablet formulations was evaluated using three dogs (two male, one female). An oral dose of 200 mg ketoprofen was administered to the dogs in a randomized cross-over study. The pellet formulations were filled into hard gelatin capsules. During administration, the capsules and the tablets were put behind the tongue to avoid destruction of the formulation due to biting. Next the formulations were ingested with a small amount of water. The time interval between each administration was at least 1 week. Twelve hours before drug administration food was withdrawn from the dogs until 24 h post-dosing, water was available ad libitum throughout the study. Blood samples (4 ml) were taken 5 min before and 0, 0.5, 1, 2, 4, 6, 8, 12 and 24 h after drug administration. Heparine (Leo Pharmaceutical Products, Zaventem, Belgium) was added as an anticoagulant. Plasma was separated by centrifugation at 2000 × *g* for 10 min and stored at −20 °C until analysis.

2.7. HPLC analysis

The plasma samples were analyzed using a modified HPLC method described by Corveleyn et al. (1996) for the determination of ketoprofen in horse plasma. A solution of 50 μg/ml naproxen (Sigma, St Louis, MO, USA) (internal standard) was prepared using the mobile phase (0.05 M pH 7.0 phosphate buffer–acetonitrile, 84:16 v/v) as a solvent. A solution of ketoprofen (Sigma, St Louis, MO, USA) at a concentration of 100 μg/ml was also prepared using the mobile phase. From this stock solution dilutions were made using the same solvent: 5, 10, 20, 30, 40 and 50 μg/ml. Calibration solutions were prepared by adding 50 μl of the naproxen solution and 50 μl of a ketoprofen solution to 450 μl of blank plasma. All calibration curves were linear over the entire concentration range ($y = 1.5950x + 0.0892$; $r^2 = 0.9995$). The plasma ketoprofen concentration in the unknown samples was calculated using a set of calibration curves ($n = 3$), obtained after linear regression of the peak area ratio (ketoprofen/naproxen) versus the ketoprofen concentration. 50 μl of the internal standard solution was added to 500 μl plasma sample. This solution was acidified by adding 1.0 ml of 1.0 M pH 2 phosphate buffer. After homogenizing and subsequent addition of 7 ml diethyl ether, the mixture was vortexed for 1 min and centrifuged for 10 min at 2000 × g. Next the organic layer was isolated and evaporated under a nitrogen stream. The residue was dissolved in 500 μl of mobile phase and a 20 μl aliquot was injected into the chromatographic system. The HPLC system (Merck, Darmstadt, Germany) consisted of an isocratic pump (LaChrom® L-7110,), a reversed-phase column protected with a LiChrocart® 4-4 guard column (both packed with LiChrospher® 5 μm 100 RP-18) and a variable wavelength UV–VIS detector (LaChrom® L-7400) set at 260 nm. The flow rate of the mobile phase was 1.5 ml/min. The column was kept at 35 °C. Drug recovery ($n = 3$) ranged between 94.6 and 98.9%. The relative standard

deviations of within-day and between-day reproducibility, calculated at different concentrations ($n = 3$), were below 4 and 7.3%, respectively.

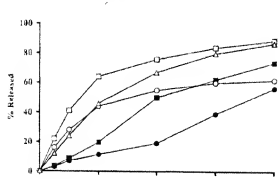

Fig. 1. In vitro dissolution profile of ketoprofen from: matrix pellets containing (□) nanocrystalline or (○) microcrystalline ketoprofen; matrix tablets containing (■) nanocrystalline or (●) microcrystalline ketoprofen; (△) Rofenid® 200 Long Acting (reference formulation). The matrix pellets contained 15% (w/w) ketoprofen (stabilized using 0.15% (w/w) sodium laurylsulphate), 35% (w/w) wax, 6.5% (w/w) drum dried corn starch and 43.5% (w/w) waxy maltodextrin. The tablets were manufactured using a binary mixture (50/50% w/w) of ketoprofen matrix pellets and placebo wax/starch pellets.

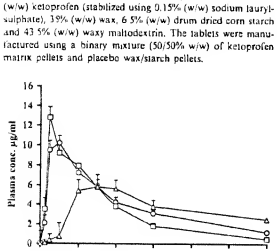

Fig. 2. Mean ($n = 6$) plasma concentrations of ketoprofen following oral administration of 200 mg ketoprofen formulated in matrix pellets: (□) nanocrystalline ketoprofen, (○) microcrystalline ketoprofen, (△) Rofenid® 200 Long Acting (reference formulation). All matrix pellet formulations contained 15% (w/w) ketoprofen (stabilized with 0.15% (w/w) sodium laurylsulphate), 35% (w/w) paraffinic wax, 6.5% (w/w) drum dried corn starch and 43.5% (w/w) waxy maltodextrin.

2.8. Pharmacokinetic analysis

The plasma concentration–time profiles were analyzed using Kinbes® software (Mediware version 3.03, Groningen, The Netherlands). The maximum ketoprofen plasma concentration (C_{max}) and the time to reach the maximum plasma concentration (t_{max}) were determined from the individual plasma concentration–time profiles. The sustained release characteristics of the ketoprofen formulations were evaluated by the $t_{75\%C_{max}}$-value. This is the time span during which the plasma concentration is at least 75% of the C_{max} value or the width of the plasma profile at 75% of C_{max}. The data were statistically evaluated using the Posthoc Sheffe test. The AUC $_{(0-24h)}$ of the plasma profiles was calculated using the logarithmic and linear trapezoidal rules.

3. Results and discussion

An important factor determining the dissolution rate and the saturation solubility, thus the bioavailability is the particle size of a drug. Micronization of drug particles has not always been successful to increase the bioavailability (Kondo et al., 1993), but further decreasing the particle size has proven to be promising (Liversidge and Cundy, 1995; Liversidge and Conzentino, 1995; Müller et al., 2001) and therefore preparing nanosuspensions was proposed as an alternative approach to increase the bioavailability of poorly soluble drugs. Vergote et al. (2001) showed that by using spray dried nanocrystalline ketoprofen matrix pellets could be prepared having a pH independent release profile, whereas the drug release from a similar dosage form formulated with microcrystalline ketoprofen was slower at lower pH of the dissolution medium. In a first bioavailability study the plasma concentration time profiles of the matrix pellets were compared with the in vivo behaviour of coated Rofenid® 200 Long Acting pellets, a commercially available formulation having a similar in vitro release profile compared with the nanocrystalline pellets (Fig. 1). As shown in Fig. 2 the in-vivo sustained release effect of the ketoprofen matrix pellets was very

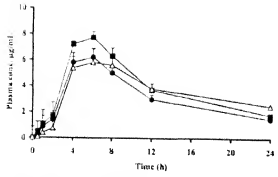

Fig. 3. Mean ($n = 3$) plasma concentrations of ketoprofen following oral administration of 200 mg ketoprofen formulated as compressed matrix pellets: (■) nanocrystalline ketoprofen, (●) microcrystalline ketoprofen, (Δ) Rofenid® 200 Long Acting (reference formulation).

limited as their $t_{75\%}$ value of the nanocrystalline ketoprofen formulation was only 1.0 h, versus 7.3 h for the reference formulation. Moreover, the matrix pellets were in-vivo characterised by a substantial burst release effect as the maximum plasma concentrations were reached within 1.0 and 2.0 h for the formulation containing nanocrystalline (12.8 μg/ml) and microcrystalline (10.2 μg/ml) ketoprofen, respectively (versus 6.0 h for the reference formulation). A possible explanation for this high initial release might be the combination of higher in vivo shear forces (Kamba et al., 2001) and the presence of a surface active agent (sodium lauryl sulphate) in the matrix pellets used as a stabilizing agent during production of the nanocrystalline suspension. It was obvious from this in-vivo experiment that the matrix pellets were not effective in formulating a sustained release dosage form and that the advantages of a pH independent in vitro drug release were not confirmed in vivo.

As the matrix pellets could easily be compressed into tablets without facing the problem of possible film damage, it was decided to run a second bioavailability experiment using compressed ketoprofen matrix pellets. These tablets were formulated using a mixture of drug loaded matrix pellets (nano- and microcrystalline) and placebo beads (based on a combination of wax, starch and disintegrant) (Vergote et al., 2002). The amount of placebo beads was optimised in order to obtain an acceptable tablet disintegration time. Partial tablet disintegration was observed at pH 1.2, whereas the tablets were fully disintegrated during in vitro dissolution experiments after 1.8 and 1.2 h at pH 4.6 and 7.5, respectively. In vitro drug release from these tablets was delayed compared with the pellets (Fig. 1). Fig. 3 shows the plasma concentration time profile following oral administration of the tablets, the bioavailability of these tablets being similar to the one of the coated reference formulation. As can been seen from Table 1, the AUC-values of the tablets were not significantly different ($P > 0.05$) from the pellet formulations, whereas t_{max} was significantly increased and C_{max} significantly reduced compared with the pellet formulations, indicating that the burst release effect was avoided. Moreover the $t_{75\%C_{max}}$ values had increased to 5.6 and 5.4 h for the nano- and microcrystalline formulation, respectively.

Table 1
Mean pharmacokinetic parameters (±SD) after oral administration of 200 mg ketoprofen to dogs

Formulation	C_{max} (μg/ml)	t_{max} (h)	$t_{75\%C_{max}}$ (h)	AUC (μg/h/ml)
Pellets ($n = 6$)				
Nanocrystalline ketoprofen	12.8 (±0.8)[a]	1.0[a]	1.0 (±0.3)[a]	94.1 (±9.5)
Microcrystalline ketoprofen	10.2 (±1.5)[a]	2.0[a]	2.2 (±0.3)[a]	86.1 (±7.0)
Rofenid® 200 Long Acting	5.8 (±0.9)	6.0	7.3 (±0.4)	86.0 (±5.5)
Compressed pellets ($n = 3$)				
Nanocrystaline ketoprofen	7.7 (±0.3)[b]	6.0[b]	5.6 (±0.6)[b]	93.2 (±6.8)
Microcrystalline ketoprofen	6.2 (±0.4)[b]	6.0[b]	5.4 (±0.5)[b]	91.4 (±7.3)

[a] Significantly different from Rofenid® 200 Long Acting (Sheffe test, $P < 0.05$).
[b] Significantly different from the corresponding pellet formulation (Sheffe test, $P < 0.05$).

From this study it was concluded that the in vivo sustained release effect of the ketoprofen matrix pellets was limited, while compressing the pellet formulations into a disintegrating tablet yielded bioavailability parameters similar to the coated pellets. The pH independent release profile observed using spray dried nanocrystalline ketoprofen was not confirmed in vivo in dogs.

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The help of R. Mouton during spray drying is gratefully acknowledged. Sincere gratitude is due to Mr. Daniël Tensy for his help during the animal experiments and to Mr. G. Masuki and A. Msami for their assistance during HPLC analysis. The authors wish to thank Eridania-Beghin Say for supplying the starch derivatives.

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EXHIBIT C

Guidance for Industry

Bioavailability and Bioequivalence Studies for Orally Administered Drug Products — General Considerations

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)
March 2003
BP

Revision 1

Guidance for Industry

Bioavailability and Bioequivalence Studies for Orally Administered Drug Products — General Considerations

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U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)
March 2003
BP

Revision 1

TABLE OF CONTENTS

Contains Nonbinding Recommendations

Guidance for Industry[1]

BA and BE Studies for Orally Administered Drug Products — General Considerations

This guidance represents the Food and Drug Administration's (FDA's) current thinking on this topic. It does not create or confer any rights for or on any person and does not operate to bind FDA or the public. An alternative approach may be used if such approach satisfies the requirements of the applicable statutes and regulations.

I. INTRODUCTION

This guidance is intended to provide recommendations to sponsors and/or applicants planning to include bioavailability (BA) and bioequivalence (BE) information for orally administered drug products in investigational new drug applications (INDs), new drug applications (NDAs), abbreviated new drug applications (ANDAs), and their supplements. This guidance contains advice on how to meet the BA and BE requirements set forth in part 320 (21 CFR part 320) as they apply to dosage forms intended for oral administration.[2] The guidance is also generally applicable to nonorally administered drug products where reliance on systemic exposure measures is suitable to document BA and BE (e.g., transdermal delivery systems and certain rectal and nasal drug products). We believe that the guidance will be useful for applicants planning to conduct BA and BE studies during the IND period for an NDA, BE studies intended for submission in an ANDA, and BE studies conducted in the postapproval period for certain changes in both NDAs and ANDAs.[3]

This guidance revises the October 2000 guidance. We have revised our recommendations regarding (1) study design and dissolution methods development, (2) comparisons of BA measures, (3) the definition of proportionality, and (4) waivers for bioequivalence studies. The guidance also makes other revisions for clarification. We believe that these revisions provide clear guidance to sponsors conducting BA and BE studies for orally administered drug products.

[2] These dosage forms include tablets, capsules, solutions, suspensions, conventional/immediate release, and modified (extended, delayed) release drug products.

[3] Other Agency guidances are available that consider specific scale-up and postapproval changes (SUPAC) for different types of drug products to help satisfy regulatory requirements in part 320 and § 314.70 (21 CFR 314.70).

FDA's guidance documents, including this guidance, do not establish legally enforceable responsibilities. Instead, guidances describe the Agency's current thinking on a topic and should be viewed only as recommendations, unless specific regulatory or statutory requirements are cited. The use of the word *should* in Agency guidances means that something is suggested or recommended, but not required.

II. BACKGROUND

A. General

Studies to measure BA and/or establish BE of a product are important elements in support of INDs, NDAs, ANDAs, and their supplements. As part of INDs and NDAs for orally administered drug products, BA studies focus on determining the process by which a drug is released from the oral dosage form and moves to the site of action. BA data provide an estimate of the fraction of the drug absorbed, as well as its subsequent distribution and elimination. BA can be generally documented by a systemic exposure profile obtained by measuring drug and/or metabolite concentration in the systemic circulation over time. The systemic exposure profile determined during clinical trials in the IND period can serve as a benchmark for subsequent BE studies.

Studies to establish BE between two products are important for certain changes before approval for a pioneer product in NDA and ANDA submissions and in the presence of certain postapproval changes in NDAs and ANDAs. In BE studies, an applicant compares the systemic exposure profile of a test drug product to that of a reference drug product (RLD). For two orally administered drug products to be bioequivalent, the active drug ingredient or active moiety in the test product must exhibit the same rate and extent of absorption as the reference drug product (see 21 CFR 320.1(e) and 320.23(b)).

Both BA and BE studies are required by regulations, depending on the type of application being submitted. Under § 314.94, BE information is required to ensure therapeutic equivalence between a pharmaceutically equivalent test drug product and a reference listed drug. Regulatory requirements for documentation of BA and BE are provided in part 320, which contains two subparts. Subpart A covers general provisions, while subpart B contains 18 sections delineating the following general BA/BE requirements:

- Requirements for submission of BA and BE data (§ 320.21)
- Criteria for waiver of an in vivo BA or BE study (§ 320.22)
- Basis for demonstrating in vivo BA or BE (§ 320.23)
- Types of evidence to establish BA or BE (§ 320.24)
- Guidelines for conduct of in vivo BA studies (§ 320.25)
- Guidelines on design of single-dose BA studies (§ 320.26)
- Guidelines on design of multiple-dose in vivo BA studies (§ 320.27)
- Correlations of BA with an acute pharmacological effect or clinical evidence (§ 320.28)

- Analytical methods for an in vivo BA study (§ 320.29)
- Inquiries regarding BA and BE requirements and review of protocols by FDA (§ 320.30)
- Applicability of requirements regarding an IND application (§ 320.31)
- Procedures for establishing and amending a BE requirement (§ 320.32)
- Criteria and evidence to assess actual or potential BE problems (§ 320.33)
- Requirements for batch testing and certification by FDA (§ 320.34)
- Requirements for in vitro batch testing of each batch (§ 320.35)
- Requirements for maintenance of records of BE testing (§ 320.36)
- Retention of BA samples (§ 320.38)
- Retention of BE samples (§ 320.63)

B. Bioavailability

Bioavailability is defined in § 320.1 as:

> the rate and extent to which the active ingredient or active moiety is absorbed from a drug product and becomes available at the site of action. For drug products that are not intended to be absorbed into the bloodstream, bioavailability may be assessed by measurements intended to reflect the rate and extent to which the active ingredient or active moiety becomes available at the site of action.

This definition focuses on the processes by which the active ingredients or moieties are released from an oral dosage form and move to the site of action.

From a pharmacokinetic perspective, BA data for a given formulation provide an estimate of the relative fraction of the orally administered dose that is absorbed into the systemic circulation when compared to the BA data for a solution, suspension, or intravenous dosage form (21 CFR 320.25(d)(2) and (3)). In addition, BA studies provide other useful pharmacokinetic information related to distribution, elimination, the effects of nutrients on absorption of the drug, dose proportionality, linearity in pharmacokinetics of the active moieties and, where appropriate, inactive moieties. BA data can also provide information indirectly about the properties of a drug substance before entry into the systemic circulation, such as permeability and the influence of presystemic enzymes and/or transporters (e.g., p-glycoprotein).

BA for orally administered drug products can be documented by developing a systemic exposure profile. A profile can be obtained by measuring the concentration of active ingredients and/or active moieties and, when appropriate, its active metabolites over time in samples collected from the systemic circulation. Systemic exposure patterns reflect both release of the drug substance from the drug product and a series of possible presystemic/systemic actions on the drug substance after its release from the drug product. We recommend that additional comparative studies be performed to understand the relative contribution of these processes to the systemic exposure pattern.

One regulatory objective is to assess, through appropriately designed BA studies, the performance of the formulations used in the clinical trials that provide evidence of safety and efficacy (21 CFR 320.25(d)(1)). Before marketing a drug product, the performance of the clinical trial dosage form can be optimized, in the context of demonstrating safety and efficacy. The systemic exposure profiles of clinical trial material can be used as a benchmark for subsequent formulation changes and can be useful as a reference for future BE studies.

Although BA studies have many pharmacokinetic objectives beyond formulation performance as described above, we note that subsequent sections of this guidance focus on using relative BA (referred to as product quality BA) and, in particular, BE studies as a means to document product quality. In vivo performance, in terms of BA/BE, can be considered to be one aspect of product quality that provides a link to the performance of the drug product used in clinical trials and to the database containing evidence of safety and efficacy.

C. Bioequivalence

Bioequivalence is defined in § 320.1 as:

> the absence of a significant difference in the rate and extent to which the active ingredient or active moiety in pharmaceutical equivalents or pharmaceutical alternatives becomes available at the site of drug action when administered at the same molar dose under similar conditions in an appropriately designed study.

As noted in the statutory definitions, both BE and product quality BA focus on the release of a drug substance from a drug product and subsequent absorption into the systemic circulation. As a result, we recommend that similar approaches to measuring BA in an NDA generally be followed in demonstrating BE for an NDA or an ANDA. Establishing product quality BA is a benchmarking effort with comparisons to an oral solution, oral suspension, or an intravenous formulation. In contrast, demonstrating BE is usually a more formal comparative test that uses specified criteria for comparisons and predetermined BE limits for such criteria.

1. IND/NDAs

BE documentation can be useful during the IND or NDA period to establish links between (1) early and late clinical trial formulations; (2) formulations used in clinical trial and stability studies, if different; (3) clinical trial formulations and to-be-marketed drug product; and (4) other comparisons, as appropriate. In each comparison, the new formulation or new method of manufacture is the test product and the prior formulation or method of manufacture is the reference product. We recommend that the determination to redocument BE during the IND period be generally left to the judgment of the sponsor, who can wish to use the principles of relevant guidances (in this guidance, see sections II.C.3, Postapproval Changes, and III.D, in Vitro Studies) to determine when changes in components, composition, and/or method of manufacture suggest further in vitro and/or in vivo studies be performed.

A test product can fail to meet BE limits because the test product has higher or lower measures of rate and extent of absorption compared to the reference product or because

the performance of the test or reference product is more variable. In some cases, nondocumentation of BE can arise because of inadequate numbers of subjects in the study relative to the magnitude of intrasubject variability, and not because of either high or low relative BA of the test product. Adequate design and execution of a BE study will facilitate understanding of the causes of nondocumentation of BE.

Where the test product generates plasma levels that are substantially above those of the reference product, the regulatory concern is not therapeutic failure, but the adequacy of the safety database from the test product. Where the test product has levels that are substantially below those of the reference product, the regulatory concern becomes therapeutic efficacy. When the variability of the test product rises, the regulatory concern relates to both safety and efficacy, because it may suggest that the test product does not perform as well as the reference product, and the test product may be too variable to be clinically useful.

Proper mapping of individual dose-response or concentration-response curves is useful in situations where the drug product has plasma levels that are either higher or lower than the reference product and are outside usual BE limits. In the absence of individual data, population dose-response or concentration-response data acquired over a range of doses, including doses above the recommended therapeutic doses, may be sufficient to demonstrate that the increase in plasma levels would not be accompanied by additional risk. Similarly, population dose- or concentration-response relationships observed over a lower range of doses, including doses below the recommended therapeutic doses, may be able to demonstrate that reduced levels of the test product compared to the reference product are associated with adequate efficacy. In either event, the burden is on the sponsor to demonstrate the adequacy of the clinical trial dose-response or concentration-response data to provide evidence of therapeutic equivalence. In the absence of this evidence, failure to document BE may suggest the product should be reformulated, the method of manufacture for the test product be changed, and/or the BE study be repeated.

2. ANDAs

BE studies are a critical component of ANDA submissions. The purpose of these studies is to demonstrate BE between a pharmaceutically equivalent generic drug product and the corresponding reference listed drug (21 CFR 314.94 (a)(7)). Together with the determination of pharmaceutical equivalence, establishing BE allows a regulatory conclusion of therapeutic equivalence.

3. Postapproval Changes

Information on the types of in vitro dissolution and in vivo BE studies that we recommend be conducted for immediate-release and modified-release drug products approved as either NDAs or ANDAs in the presence of specified postapproval changes is provided in the FDA guidances for industry entitled *SUPAC-IR: Immediate Release Solid Oral Dosage Forms: Scale-Up and Post-Approval Changes: Chemistry, Manufacturing, and Controls, In Vitro Dissolution Testing, and In Vivo Bioequivalence*

Documentation, and *SUPAC-MR: Modified Release Solid Oral Dosage Forms: Scale-Up and Post-Approval Changes: Chemistry, Manufacturing, and Controls, In Vitro Dissolution Testing, and In Vivo Bioequivalence Documentation*. In the presence of certain major changes in components, composition, and/or method of manufacture after approval, we recommend that in vivo BE be redemonstrated. For approved NDAs, we also recommend that the drug product after the change be compared to the drug product before the change. For approved ANDAs, we also recommend that the drug product after the change be compared to the reference listed drug. Under section 506A(c)(2)(B) of the Federal Food, Drug, and Cosmetic Act (the Act) (21 U.S.C. 356a(c)(2)(B)), postapproval changes requiring completion of studies in accordance with part 320 must be submitted in a supplement and approved by FDA before distributing a drug product made with the change.

III. METHODS TO DOCUMENT BA AND BE

As noted in § 320.24, several in vivo and in vitro methods can be used to measure product quality BA and to establish BE. In descending order of preference, these include pharmacokinetic, pharmacodynamic, clinical, and in vitro studies. These general approaches are discussed in the following sections of this guidance. Product quality BA and BE frequently rely on pharmacokinetic measures such as AUC and Cmax that are reflective of systemic exposure.

A. Pharmacokinetic Studies

1. General Considerations

The statutory definitions of BA and BE, expressed in terms of rate and extent of absorption of the active ingredient or moiety to the site of action, emphasize the use of pharmacokinetic measures in an accessible biological matrix such as blood, plasma, and/or serum to indicate release of the drug substance from the drug product into the systemic circulation.[4] This approach rests on an understanding that measuring the active moiety or ingredient at the site of action is generally not possible and, furthermore, that some relationship exists between the efficacy/safety and concentration of active moiety and/or its important metabolite or metabolites in the systemic circulation. To measure product quality BA and establish BE, reliance on pharmacokinetic measurements may be viewed as a bioassay that assesses release of the drug substance from the drug product into the systemic circulation. A typical study is conducted as a crossover study. In this type of study, clearance, volume of distribution, and absorption, as determined by physiological variables (e.g. gastric emptying, motility, pH), are assumed to have less interoccasion variability compared to the variability arising from formulation performance. Therefore, differences between two products because of formulation factors can be determined.

[4] If serial measurements of the drug or its metabolites in plasma, serum, or blood cannot be accomplished, measurement of urinary excretion can be used to document BE.

2. Pilot Study

If the sponsor chooses, a pilot study in a small number of subjects can be carried out before proceeding with a full BE study. The study can be used to validate analytical methodology, assess variability, optimize sample collection time intervals, and provide other information. For example, for conventional immediate-release products, careful timing of initial samples may avoid a subsequent finding in a full-scale study that the first sample collection occurs after the plasma concentration peak. For modified-release products, a pilot study can help determine the sampling schedule to assess lag time and dose dumping. A pilot study that documents BE can be appropriate, provided its design and execution are suitable and a sufficient number of subjects (e.g., 12) have completed the study.

3. Pivotal Bioequivalence Studies

General recommendations for a standard BE study based on pharmacokinetic measurements are provided in Attachment A.

4. Study Designs

Nonreplicate crossover study designs are recommended for BE studies of immediate-release and modified-release dosage forms. However, sponsors and/or applicants have the option of using replicate designs for BE studies for these drug products. Replicate study designs may offer several scientific advantages compared to nonreplicate designs. The advantages of replicate study designs may be that they (1) allow comparisons of within-subject variances for the test and reference products, (2) provide more information about the intrinsic factors underlying formulation performance, and (3) reduce the number of subjects participating in the BE study. The recommended method of analysis of nonreplicate or replicate studies to establish BE is average bioequivalence, as discussed in section IV. General recommendations for nonreplicate study designs are provided in Attachment A. Recommendations for replicate study designs can be found in the guidance for industry *Statistical Approaches to Establishing Bioequivalence.*

5. Study Population

We recommend that, unless otherwise indicated by a specific guidance, subjects recruited for in vivo BE studies be 18 years of age or older and capable of giving informed consent. This guidance recommends that in vivo BE studies be conducted in individuals representative of the general population, taking into account age, sex, and race. We recommend that if the drug product is intended for use in both sexes, the sponsor attempt to include similar proportions of males and females in the study. If the drug product is to be used predominantly in the elderly, we also recommend that the sponsor attempt to include as many subjects of 60 years of age or older as possible. We recommend that the total number of subjects in the study provide adequate power for BE demonstration, but it is not expected that there will be sufficient power to draw conclusions for each subgroup.

Statistical analysis of subgroups is not recommended. We recommend that restrictions on admission into the study generally be based solely on safety considerations. In some instances, it may be useful to admit patients into BE studies for whom a drug product is intended. In this situation, we recommend that sponsors and/or applicants attempt to enter patients whose disease process is stable for the duration of the BE study. In accordance with § 320.31, for some products that will be submitted in ANDAs, an IND may be required for BE studies to ensure patient safety.

6. Single-Dose/Multiple-Dose Studies

Instances where multiple-dose studies can be useful are defined under § 320.27(a)(3). However, this guidance generally recommends single-dose pharmacokinetic studies for both immediate- and modified-release drug products to demonstrate BE because they are *generally* more sensitive in assessing release of the drug substance from the drug product into the systemic circulation (see section V). We recommend that if a multiple-dose study design is important, appropriate dosage administration and sampling be carried out to document attainment of steady state.

7. Bioanalytical Methodology

We recommend sponsors ensure that bioanalytical methods for BA and BE studies are accurate, precise, selective, sensitive, and reproducible. A separate FDA guidance entitled *Bioanalytical Method Validation* is available to assist sponsors in validating bioanalytical methods.

8. Pharmacokinetic Measures of Systemic Exposure

Both direct (e.g., rate constant, rate profile) and indirect (e.g., Cmax, Tmax, mean absorption time, mean residence time, Cmax normalized to AUC) pharmacokinetic measures are limited in their ability to assess rate of absorption. This guidance, therefore, recommends a change in focus from these direct or indirect measures of absorption rate to measures of systemic exposure. Cmax and AUC can continue to be used as measures for product quality BA and BE, but more in terms of their capacity to assess exposure than their capacity to reflect rate and extent of absorption. We recommend that reliance on systemic exposure measures reflect comparable rate and extent of absorption, which in turn would achieve the underlying statutory and regulatory objective of ensuring comparable therapeutic effects. Exposure measures are defined relative to early, peak, and total portions of the plasma, serum, or blood concentration-time profile, as follows:

a. Early Exposure

For orally administered immediate-release drug products, BE can generally be demonstrated by measurements of peak and total exposure. An early exposure measure may be informative on the basis of appropriate clinical efficacy/safety trials and/or pharmacokinetic/pharmacodynamic studies that call for better control of drug absorption into the systemic circulation (e.g., to ensure rapid onset of an

analgesic effect or to avoid an excessive hypotensive action of an antihypertensive). In this setting, the guidance recommends use of partial AUC as an early exposure measure. We recommend that the partial area be truncated at the population median of Tmax values for the reference formulation. We also recommend that at least two quantifiable samples be collected before the expected peak time to allow adequate estimation of the partial area.

b. Peak Exposure

We recommend that peak exposure be assessed by measuring the peak drug concentration (Cmax) obtained directly from the data without interpolation.

c. Total Exposure

For single-dose studies, we recommend that the measurement of total exposure be:

- Area under the plasma/serum/blood concentration-time curve from time zero to time t (AUC_{0-t}), where t is the last time point with measurable concentration for individual formulation.

- Area under the plasma/serum/blood concentration-time curve from time zero to time infinity ($AUC_{0-\infty}$), where $AUC_{0-\infty} = AUC_{0-t} + C_t/\lambda_z$, C_t is the last measurable drug concentration and λ_z is the terminal or elimination rate constant calculated according to an appropriate method. We recommend that the terminal half-life ($t_{1/2}$) of the drug also be reported.

For steady-state studies, we recommend that the measurement of total exposure be the area under the plasma, serum, or blood concentration-time curve from time zero to time tau over a dosing interval at steady state (AUC_{0-tau}), where tau is the length of the dosing interval.

B. Pharmacodynamic Studies

Pharmacodynamic studies are not recommended for orally administered drug products when the drug is absorbed into the systemic circulation and a pharmacokinetic approach can be used to assess systemic exposure and establish BE. However, in those instances where a pharmacokinetic approach is not possible, suitably validated pharmacodynamic methods can be used to demonstrate BE.

C. Comparative Clinical Studies

Where there are no other means, well-controlled clinical trials in humans can be useful to provide supportive evidence of BA or BE. However, we recommend that the use of comparative clinical trials as an approach to demonstrate BE generally be considered insensitive and be avoided where possible (21 CFR 320.24). The use of BE studies with clinical trial endpoints can

be appropriate to demonstrate BE for orally administered drug products when measurement of the active ingredients or active moieties in an accessible biological fluid (pharmacokinetic approach) or pharmacodynamic approach is infeasible.

D. In Vitro Studies

Under certain circumstances, product quality BA and BE can be documented using in vitro approaches (21 CFR 320.24(b)(5) and 21 CFR 320.22(d)(3)). For highly soluble, highly permeable, rapidly dissolving, and orally administered drug products, documentation of BE using an in vitro approach (dissolution studies) is appropriate based on the biopharmaceutics classification system.[5] This approach may also be suitable under some circumstances in assessing BE during the IND period, for NDA and ANDA submissions, and in the presence of certain postapproval changes to approved NDAs and ANDAs. In addition, in vitro approaches to documenting BE for *nonbioproblem* drugs approved before 1962 remain appropriate (21 CFR 320.33).

Dissolution testing is also used to assess batch-to-batch quality, where the dissolution tests, with defined procedures and acceptance criteria, are used to allow batch release. We recommend that dissolution testing is also used to (1) provide process control and quality assurance, and (2) assess whether further BE studies relative to minor postapproval changes be conducted, where dissolution can function as a signal of bioinequivalence. In vitro dissolution characterization is encouraged for all product formulations investigated (including prototype formulations), particularly if in vivo absorption characteristics are being defined for the different product formulations. Such efforts may enable the establishment of an in vitro-in vivo correlation. When an in vitro-in vivo correlation or association is available (21 CFR 320.24(b)(1)(ii)), the in vitro test can serve not only as a quality control specification for the manufacturing process, but also as an indicator of how the product will perform in vivo. The following guidances provide recommendations on the development of dissolution methodology, setting specifications, and the regulatory applications of dissolution testing: (1) *Dissolution Testing of Immediate Release Solid Oral Dosage Forms*; and (2) *Extended Release Oral Dosage Forms: Development, Evaluation, and Application of In Vitro/In Vivo Correlations.*

We recommend that the following information generally be included in the dissolution method development report for solid oral dosage forms:

For an NDA:

- The pH solubility profile of the drug substance
- Dissolution profiles generated at different agitation speeds (e.g., 100 to 150 revolutions per minute (rpm) for U.S. Pharmacopeia (USP) Apparatus I (basket), or 50 to 100 rpm for USP Apparatus II (paddle))

[5] See the FDA guidance for industry on *Waiver of In Vivo Bioavailability and Bioequivalence Studies for Immediate Release Solid Oral Dosage Forms Based on a Biopharmaceutics Classification System*. This document provides complementary information on the Biopharmaceutics Classification System (BCS).

- Dissolution profiles generated on all strengths in at least three dissolution media (e.g., pH 1.2, 4.5, and 6.8 buffer). Water can be used as an additional medium. If the drug being considered is poorly soluble, appropriate concentrations of surfactants are recommended.

It is recommended that the sponsor select the agitation speed and medium that provide adequate discriminating ability, taking into account all the available in vitro and in vivo data.

For ANDAs:

- For immediate-release drug products, we recommend that the appropriate USP method be submitted. If there is no USP method available, we recommend that the FDA method for the reference listed drug be used. If the USP and/or FDA methods are not available, we recommend that the dissolution method development report described above be submitted.
- For modified-release products, dissolution profiles using the appropriate USP method (if available) can be submitted. If there is no USP method available, we recommend that the FDA method for the reference listed drug be used. In addition, we recommend that profiles using at least three other dissolution media (e.g., pH 1.2, 4.5, and 6.8 buffer) and water be submitted.

This guidance recommends that dissolution data from three batches for both NDAs and ANDAs be used to set dissolution specifications for modified-release dosage forms, including extended-release dosage forms.

IV. COMPARISON OF BA MEASURES IN BE STUDIES

An equivalence approach has been and continues to be recommended for BE comparisons. The recommended approach relies on (1) a criterion to allow the comparison, (2) a confidence interval for the criterion, and (3) a BE limit. Log-transformation of exposure measures before statistical analysis is recommended. BE studies are performed as single-dose, crossover studies. To compare measures in these studies, data have been analyzed using an average BE criterion. This guidance recommends continued use of an average BE criterion to compare BA measures for replicate and nonreplicate BE studies of both immediate- and modified-release products.

V. DOCUMENTATION OF BA AND BE

An in vivo study is generally recommended for all solid oral dosage forms approved after 1962 and for *bioproblem* drug products approved before 1962. Waiver of in vivo studies for different strengths of a drug product can be granted under § 320.22(d)(2) when (1) the drug product is in the same dosage form, but in a different strength; (2) this different strength is *proportionally similar* in its active and inactive ingredients to the strength of the product for which the same manufacturer has conducted an appropriate in vivo study; and (3) the new strength meets an

appropriate in vitro dissolution test. This guidance defines *proportionally similar* in the following ways:

- All active and inactive ingredients are in exactly the same proportion between different strengths (e.g., a tablet of 50-mg strength has all the inactive ingredients, exactly half that of a tablet of 100-mg strength, and twice that of a tablet of 25-mg strength).

- Active and inactive ingredients are not in exactly the same proportion between different strengths as stated above, but the ratios of inactive ingredients to total weight of the dosage form are within the limits defined by the SUPAC-IR and SUPAC-MR guidances up to and including Level II.

- For high potency drug substances, where the amount of the active drug substance in the dosage form is relatively low, the total weight of the dosage form remains nearly the same for all strengths (within ± 10 % of the total weight of the strength on which a biostudy was performed), the same inactive ingredients are used for all strengths, and the change in any strength is obtained by altering the amount of the active ingredients and one or more of the inactive ingredients. The changes in the inactive ingredients are within the limits defined by the SUPAC-IR and SUPAC-MR guidances up to and including Level II.

Exceptions to the above definitions may be possible, if adequate justification is provided.

A. Solutions

For oral solutions, elixirs, syrups, tinctures, or other solubilized forms, in vivo BA and/or BE can be waived (21 CFR 320.22(b)(3)(i)). Generally, in vivo BE studies are waived for solutions on the assumption that release of the drug substance from the drug product is self-evident and that the solutions do not contain any excipient that significantly affects drug absorption (21 CFR 320.22(b)(3)(iii)). However, there are certain excipients, such as sorbitol or mannitol, that can reduce the bioavailability of drugs with low intestinal permeability in amounts sometimes used in oral liquid dosage forms.

B. Suspensions

We recommend that BA and BE for a suspension generally be established for immediate-release solid oral dosage forms, and both in vivo and in vitro studies are recommended.

C. Immediate-Release Products: Capsules and Tablets

1. General Recommendations

For product quality BA and BE studies, we recommend that where the focus is on release of the drug substance from the drug product into the systemic circulation, a single-dose, fasting study be performed. We also recommend that in vivo BE studies be accompanied

by in vitro dissolution profiles on all strengths of each product. For ANDAs, we also recommend that the BE study be conducted between the test product and reference listed drug using the strength(s) specified in *Approved Drug Products with Therapeutic Equivalence Evaluations (Orange Book)*.

2. *Waivers of In Vivo BE Studies (Biowaivers)*

   a. INDs, NDAs, and ANDAs: Preapproval

   When the drug product is in the same dosage form, but in a different strength, and is proportionally similar in its active and inactive ingredients to the strength on which BA or BE testing has been conducted, an in vivo BE demonstration of one or more lower strengths can be waived based on dissolution tests and an in vivo study on the highest strength.[8]

   For an NDA, biowaivers of a higher strength will be determined to be appropriate based on (1) clinical safety and/or efficacy studies including data on the dose and the desirability of the higher strength, (2) linear elimination kinetics over the therapeutic dose range, (3) the higher strength being proportionally similar to the lower strength, and (4) the same dissolution procedures being used for both strengths and similar dissolution results obtained. We recommend that a dissolution profile be generated for all strengths.

   If an appropriate dissolution method has been established (see section III.D.), and the dissolution results indicate that the dissolution characteristics of the product are not dependent on the product strength, then dissolution profiles in one medium are usually sufficient to support waivers of in vivo testing. Otherwise, dissolution data in three media (pH 1.2, 4.5, and 6.8) are recommended. We recommend that the f_2 test be used to compare profiles from the different strengths of the product. An f_2 value ≥ 50 indicates a sufficiently similar dissolution profile such that further in vivo studies are not needed. For an f_2 value < 50, further discussions with CDER review staff may help to determine whether an in vivo study is appropriate (21 CFR 320.22(d)(2)(ii)). The f_2 approach is not suitable for rapidly dissolving drug products (e.g., $\geq$ 85% dissolved in 15 minutes or less).

   For an ANDA, conducting an in vivo study on a strength that is not the highest may be appropriate for reasons of safety, subject to approval by the Division of Bioequivalence, Office of Generic Drugs, and provided that the following conditions are met:

   - Linear elimination kinetics has been shown over the therapeutic dose range.

[8] This recommendation modifies a prior policy of allowing biowaivers for only three lower strengths on ANDAs.

- The higher strengths of the test and reference products are proportionally similar to their corresponding lower strength.

- Comparative dissolution testing on the higher strength of the test and reference products is submitted and found to be appropriate.

b. NDAs and ANDAs: Postapproval

Information on the types of in vitro dissolution and in vivo BE studies for immediate-release drug products approved as either NDAs or ANDAs in the presence of specified postapproval changes are provided in an FDA guidance for industry entitled *SUPAC-IR: Immediate Release Solid Oral Dosage Forms: Scale-Up and Post-Approval Changes: Chemistry, Manufacturing, and Controls, In Vitro Dissolution Testing, and In Vivo Bioequivalence Documentation*. For postapproval changes, we recommend that the in vitro comparison be made between the prechange and postchange products. In instances where dissolution profile comparisons are suggested, we also recommend an f_2 test be used. An f_2 value of ≥ 50 suggests a sufficiently similar dissolution profile and no further in vivo studies are needed. When in vivo BE studies are called for, we recommend that the comparison be made for NDAs between the prechange and postchange products, and for ANDAs between the postchange and reference listed drug products.

D. Modified-Release Products

Modified-release products include delayed-release products and extended- (controlled) release products.

As defined in the USP, delayed-release drug products are dosage forms that release the drugs at a time later than immediately after administration (i.e., these drug products exhibit a lag time in quantifiable plasma concentrations). Typically, coatings (e.g., enteric coatings) are intended to delay the release of medication until the dosage form has passed through the acidic medium of the stomach. In vivo tests for delayed-release drug products are similar to those for extended-release drug products. We recommend that in vitro dissolution tests for these products document that they are stable under acidic conditions and that they release the drug only in a neutral medium (e.g., pH 6.8).

Extended-release drug products are dosage forms that allow a reduction in dosing frequency as compared to when the drug is present in an immediate-release dosage form. These drug products can be developed to reduce fluctuations in plasma concentrations. Extended-release products can be capsules, tablets, granules, pellets, and suspensions. If any part of a drug product includes an extended-release component, the following recommendations apply.

1. NDAs: BA and BE Studies

An NDA can be submitted for a previously unapproved new molecular entity, new salt, new ester, prodrug, or other noncovalent derivative of a previously approved new molecular entity formulated as a modified-release drug product. We recommend that the first modified-release drug product for a previously approved immediate-release drug product be submitted as an NDA. We also recommend that subsequent modified-release products that are pharmaceutically equivalent and bioequivalent to the listed drug product be submitted as ANDAs. BA requirements for the NDA of an extended-release product are listed in § 320.25(f). The purpose of an in vivo BA study for which a controlled-release claim is made is to determine if all of the following conditions are met:

- The drug product meets the controlled-release claims made for it.
- The BA profile established for the drug product rules out the occurrence of any dose dumping.
- The drug product's steady-state performance is equivalent to a currently marketed noncontrolled release or controlled-release drug product that contains the same active drug ingredient or therapeutic moiety and that is subject to an approved full NDA.
- The drug product's formulation provides consistent pharmacokinetic performance between individual dosage units.

As noted in § 320.25(f)(2), "the reference material(s) for such a bioavailability study shall be chosen to permit an appropriate scientific evaluation of the controlled release claims made for the drug product," such as:

- A solution or suspension of the active drug ingredient or therapeutic moiety
- A currently marketed noncontrolled-release drug product containing the same active drug ingredient or therapeutic moiety and administered according to the dosage recommendations in the labeling
- A currently marketed controlled-release drug product subject to an approved full NDA containing the same active drug ingredient or therapeutic moiety and administered according to the dosage recommendations in the labeling

This guidance recommends that the following BA studies be conducted for an extended-release drug product submitted as an NDA:

- A single-dose, fasting study on all strengths of tablets and capsules and highest strength of beaded capsules
- A single-dose, food-effect study on the highest strength

- A steady-state study on the highest strength

BE studies are recommended when substantial changes in the components or composition and/or method of manufacture for an extended-release drug product occur between the to-be-marketed NDA dosage form and the clinical trial material.

2. *ANDAs: BE Studies*

For modified-release products submitted as ANDAs, the following studies are recommended: (1) a single-dose, nonreplicate, fasting study comparing the highest strength of the test and reference listed drug product and (2) a food-effect, nonreplicate study comparing the highest strength of the test and reference product (see section VI.A). Because single-dose studies are considered more sensitive in addressing the primary question of BE (i.e., release of the drug substance from the drug product into the systemic circulation), multiple-dose studies are generally not recommended, even in instances where nonlinear kinetics are present.

3. *Waivers of In Vivo BE Studies (Biowaivers): NDAs and ANDAs*

a. Beaded Capsules — Lower Strength

We recommend that for modified-release beaded capsules where the strength differs only in the number of beads containing the active moiety, a single-dose, fasting BE study be carried out only on the highest strength, with waiver of in vivo studies for lower strengths based on dissolution profiles. A dissolution profile can be generated for each strength using the recommended dissolution method. The f_2 test can be used to compare profiles from the different strengths of the product. An f_2 value of ≥ 50 can be used to confirm that further in vivo studies are not needed.

b. Tablets — Lower Strength

For modified-release tablets, when the drug product is in the same dosage form but in a different strength, when it is proportionally similar in its active and inactive ingredients, and when it has the same drug release mechanism, an in vivo BE determination of one or more lower strengths can be waived based on dissolution profile comparisons, with an in vivo study only on the highest strength. We recommend that the drug products exhibit similar dissolution profiles between the highest strength and the lower strengths based on the f_2 test in at least three dissolution media (e.g., pH 1.2, 4.5 and 6.8). We recommend that the dissolution profile be generated on the test and reference products of all strengths.

4. *Postapproval Changes*

Information on the types of in vitro dissolution and in vivo BE studies for extended-release drug products approved as either NDAs or ANDAs in the presence of specified postapproval changes are provided in an FDA guidance for industry entitled *SUPAC-MR: Modified Release Solid Oral Dosage Forms: Scale-Up and Post-Approval Changes: Chemistry, Manufacturing, and Controls, In Vitro Dissolution Testing, and In Vivo Bioequivalence Documentation*. We recommend that for postapproval changes, the in vitro comparison be made between the prechange and postchange products. In instances where dissolution profile comparisons are recommended, an f_2 test can be used. An f_2 value of ≥ 50 suggests a similar dissolution profile. A failure to demonstrate similar dissolution profiles may indicate an in vivo BE study be performed. When in vivo BE studies are conducted, we recommend that the comparison be made for NDAs between the prechange and postchange products, and for ANDAs between the postchange product and reference listed drug.

E. Miscellaneous Dosage Forms

We recommend that rapidly dissolving drug products, such as buccal and sublingual dosage forms (and chewable tablets), be tested for in vitro dissolution and in vivo BA and/or BE. We recommend that chewable tablets (as a whole) be subject to in vitro dissolution testing because they might be swallowed by a patient without proper chewing. In general, we recommend that in vitro dissolution test conditions for chewable tablets be the same as for nonchewable tablets of the same active ingredient or moiety. Infrequently, different test conditions or acceptance criteria can be indicated for chewable and nonchewable tablets, but we recommend these differences, if they exist, be resolved with the appropriate review division.

VI. SPECIAL TOPICS

A. Food-Effect Studies

Co-administration of food with oral drug products may influence drug BA and/or BE. Food-effect BA studies focus on the effects of food on the release of the drug substance from the drug product as well as the absorption of the drug substance. BE studies with food focus on demonstrating comparable BA between test and reference products when coadministered with meals. Usually, a single-dose, two-period, two-treatment, two-sequence crossover study is recommended for both food-effect BA and BE studies.

B. Moieties to Be Measured

1. Parent Drug Versus Metabolites

The moieties to be measured in biological fluids collected in BA and BE studies are either the active drug ingredient or its active moiety in the administered dosage form

(parent drug) and, when appropriate, its active metabolites (21 CFR 320.24(b)(1)(ii)).[9] This guidance recommends the following approaches for BA and BE studies.

For BA studies (see section II.B), we recommend that determination of moieties to be measured in biological fluids take into account both concentration and activity. *Concentration* refers to the relative quantity of the parent drug or one or more metabolites in a given volume of an accessible biological fluid such as blood or plasma. *Activity* refers to the relative contribution of the parent drug and its metabolite(s) in the biological fluids to the clinical safety and/or efficacy of the drug. For BA studies, we also recommend that both the parent drug and its major active metabolites be measured, if analytically feasible.

For BE studies, measurement of only the parent drug released from the dosage form, rather than the metabolite, is generally recommended. The rationale for this recommendation is that concentration-time profile of the parent drug is more sensitive to changes in formulation performance than a metabolite, which is more reflective of metabolite formation, distribution, and elimination. The following are exceptions to this general approach.

- Measurement of a metabolite may be preferred when parent drug levels are too low to allow reliable analytical measurement in blood, plasma, or serum for an adequate length of time. We recommend that the metabolite data obtained from these studies be subject to a confidence interval approach for BE demonstration. If there is a clinical concern related to efficacy or safety for the parent drug, we also recommend that sponsors and/or applicants contact the appropriate review division to determine whether the parent drug should be measured and analyzed statistically.

- A metabolite may be formed as a result of gut wall or other presystemic metabolism. If the metabolite contributes meaningfully to safety and/or efficacy, we also recommend that the metabolite and the parent drug be measured. When the relative activity of the metabolite is low and does not contribute meaningfully to safety and/or efficacy, it does not have to be measured. We recommend that the parent drug measured in these BE studies be analyzed using a confidence interval approach. The metabolite data can be used to provide supportive evidence of comparable therapeutic outcome.

2. *Enantiomers Versus Racemates*

For BA studies, measurement of individual enantiomers may be important. For BE studies, this guidance recommends measurement of the racemate using an achiral assay. Measurement of individual enantiomers in BE studies is recommended only when all of

[9] A dosage form contains active and, usually, inactive ingredients. The active ingredient may be a prodrug that becomes active with further in vivo transformation. An active moiety is the molecule or ion, excluding those appended portions of the molecule that cause the drug to be an ester, salt, or other noncovalent derivative of the molecule, responsible for the physiological or pharmacological action of the drug substance.

the following conditions are met: (1) the enantiomers exhibit different pharmacodynamic characteristics, (2) the enantiomers exhibit different pharmacokinetic characteristics, (3) primary efficacy and safety activity resides with the minor enantiomer, and (4) nonlinear absorption is present (as expressed by a change in the enantiomer concentration ratio with change in the input rate of the drug) for at least one of the enantiomers. In such cases, we recommend that BE factors be applied to the enantiomers separately.

3. Drug Products With Complex Mixtures as the Active Ingredients

Certain drug products may contain complex drug substances (i.e., active moieties or active ingredients that are mixtures of multiple synthetic and/or natural source components). Some or all of the components of these complex drug substances cannot be characterized with regard to chemical structure and/or biological activity. Quantification of all active or potentially active components in pharmacokinetic studies to document BA and BE is neither encouraged nor desirable. Rather, we recommend that BA and BE studies be based on a small number of markers of rate and extent of absorption. Although a case-by-case determination, criteria for marker selection include amount of the moiety in the dosage form, plasma or blood levels of the moiety, and biological activity of the moiety relative to other moieties in the complex mixture. Where pharmacokinetic approaches are infeasible to assess rate and extent of absorption of a drug substance from a drug product, in vitro approaches may be preferred. Pharmacodynamic or clinical approaches may be called for if no quantifiable moieties are available for in vivo pharmacokinetic or in vitro studies.

C. Long Half-Life Drugs

In a BA or pharmacokinetic study involving an oral product with a long half-life drug, adequate characterization of the half-life calls for blood sampling over a long period of time. For a BE determination of an oral product with a long half-life drug, a nonreplicate, single-dose, crossover study can be conducted, provided an adequate washout period is used. If the crossover study is problematic, a BE study with a parallel design can be used. For either a crossover or parallel study, we recommend that sample collection time be adequate to ensure completion of gastrointestinal transit (approximately 2 to 3 days) of the drug product and absorption of the drug substance. Cmax and a suitably truncated AUC can be used to characterize peak and total drug exposure, respectively. For drugs that demonstrate low intrasubject variability in distribution and clearance, an AUC truncated at 72 hours ($AUC_{0\text{-}72\ hr}$) can be used in place of $AUC_{0\text{-}t}$ or $AUC_{0\text{-}\infty}$. For drugs demonstrating high intrasubject variability in distribution and clearance, AUC truncation warrants caution. In such cases, we also recommend that sponsors and/or applicants consult the appropriate review staff.

D. First Point Cmax

The first point of a concentration-time curve in a BE study based on blood and/or plasma measurements is sometimes the highest point, which raises a question about the measurement of true Cmax because of insufficient early sampling times. A carefully conducted pilot study may avoid this problem. Collection of an early time point between 5 and 15 minutes after dosing

followed by additional sample collections (e.g., two to five) in the first hour after dosing may be sufficient to assess early peak concentrations. If this sampling approach is followed, we recommend that data sets be considered adequate, even when the highest observed concentration occurs at the first time point.

E. Orally Administered Drugs Intended for Local Action

Documentation of product quality BA for NDAs where the drug substance produces its effects by local action in the gastrointestinal tract can be achieved using clinical efficacy and safety studies and/or suitably designed and validated in vitro studies. Similarly, documentation of BE for ANDAs, and for both NDAs and ANDAs in the presence of certain postapproval changes, can be achieved using BE studies with clinical efficacy and safety endpoints and/or suitably designed and validated in vitro studies, if the latter studies are either reflective of important clinical effects or are more sensitive to changes in product performance compared to a clinical study. To ensure comparable safety, additional studies with and without food may help to understand the degree of systemic exposure that occurs following administration of a drug product intended for local action in the gastrointestinal tract.

F. Narrow Therapeutic Range Drugs

This guidance defines *narrow therapeutic range*[10] drug products as containing certain drug substances subject to therapeutic drug concentration or pharmacodynamic monitoring, and/or where product labeling indicates a narrow therapeutic range designation. Examples include digoxin, lithium, phenytoin, theophylline, and warfarin. Because not all drugs subject to therapeutic drug concentration or pharmacodynamic monitoring are narrow therapeutic range drugs, sponsors and/or applicants can contact the appropriate review division at CDER to determine whether a drug can or cannot be considered to have a narrow therapeutic range.

This guidance recommends that sponsors consider additional testing and/or controls to ensure the quality of drug products containing narrow therapeutic range drugs. The approach is designed to provide increased assurance of interchangeability for drug products containing specified narrow therapeutic range drugs. It is not designed to influence the practice of medicine or pharmacy.

Unless otherwise indicated by a specific guidance, this guidance recommends that the traditional BE limit of 80 to 125 percent for non-narrow therapeutic range drugs remain unchanged for the bioavailability measures (AUC and Cmax) of narrow therapeutic range drugs.

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[10] This guidance uses the term *narrow therapeutic range* instead of *narrow therapeutic index* drug, although the latter is more commonly used.

ATTACHMENT: GENERAL PHARMACOKINETIC STUDY DESIGN AND DATA HANDLING

For both replicate and nonreplicate, in vivo pharmacokinetic BE studies, the following general approaches are recommended, recognizing that the elements can be adjusted for certain drug substances and drug products.

Study conduct:

- The test or reference products can be administered with about 8 ounces (240 milliliters) of water to an appropriate number of subjects under fasting conditions, unless the study is a food-effect BA and BE study.

- Generally, the highest marketed strength can be administered as a single unit. If warranted for analytical reasons, multiple units of the highest strength can be administered, providing the total single-dose remains within the labeled dose range.

- An adequate washout period (e.g., more than 5 half lives of the moieties to be measured) would separate each treatment.

- The lot numbers of both test and reference listed products and the expiration date for the reference product would be stated. The drug content of the test product cannot differ from that of the reference listed product by more than 5 percent. The sponsor can include a statement of the composition of the test product and, if possible, a side-by-side comparison of the compositions of test and reference listed products. In accordance with § 320.38, samples of the test and reference listed product must be retained for 5 years.

- Before and during each study phase, we recommend that subjects (1) be allowed water as desired except for 1 hour before and after drug administration, (2) be provided standard meals no less than 4 hours after drug administration, and (3) abstain from alcohol for 24 hours before each study period and until after the last sample from each period is collected.

Sample collection and sampling times:

- We recommend that under normal circumstances, blood, rather than urine or tissue, be used. In most cases, drug, or metabolites are measured in serum or plasma. However, in certain cases, whole blood may be more appropriate for analysis. We recommend that blood samples be drawn at appropriate times to describe the absorption, distribution, and elimination phases of the drug. For most drugs, we recommend that 12 to 18 samples, including a predose sample, be collected per subject per dose. This sampling can continue for at least three or more terminal half lives of the drug. The exact timing for sample collection depends on the nature of the drug and the input from the administered dosage form. The sample collection can be spaced in such a way that the maximum concentration of the drug in the blood (Cmax) and terminal elimination rate constant (λ_z) can be estimated accurately. At least three to four samples can be obtained during the terminal log-linear phase to obtain an accurate estimate of λ_z from linear regression. We recommend that the actual clock time when samples are drawn as well as the elapsed time related to drug administration be recorded.

Subjects with predose plasma concentrations:

- If the predose concentration is ≤ 5 percent of Cmax value in that subject, the subject's data without any adjustments can be included in all pharmacokinetic measurements and calculations. We recommend that if the predose value is > than 5 percent of Cmax, the subject be dropped from all BE study evaluations.

Data deletion due to vomiting:

- We recommend that data from subjects who experience emesis during the course of a BE study for immediate-release products be deleted from statistical analysis if vomiting occurs at or before 2 times median Tmax. In the case of modified-release products, the data from subjects who experience emesis any time during the labeled dosing interval can be deleted.

The following pharmacokinetic information is recommended for submission:

- Plasma concentrations and time points
- Subject, period, sequence, treatment
- AUC_{0-t}, $AUC_{0-\infty}$, Cmax, Tmax, λ_z, and $t_{1/2}$
- Intersubject, intrasubject, and/or total variability, if available
- Cmin (concentration at the end of a dosing interval), Cav (average concentration during a dosing interval), degree of fluctuation [(Cmax-Cmin)/Cav], and swing [(Cmax-Cmin)/Cmin] if steady-state studies are employed
- Partial AUC, requested only as discussed in section III. A.9.a.

In addition, we recommend that the following statistical information be provided for AUC_{0-t}, $AUC_{0-\infty}$, and Cmax:

- Geometric mean
- Arithmetic mean
- Ratio of means
- Confidence intervals

We also recommend that logarithmic transformation be provided for measures used for BE demonstration.

Rounding off of confidence interval values:

- We recommend that confidence interval (CI) values not be rounded off; therefore, to pass a CI limit of 80 to125, the value would be at least 80.00 and not more than 125.00.

EXHIBIT D

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NANOSUSPENSION: AN ATTEMPT TO ENHANCE BIOAVAILABILITY OF POORLY SOLUBLE DRUGS

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ABSTRACT

Most of the new chemical entities coming out from High-throughput screening in drug discovery process are failing due to their poor solubility in the water. Poorly water-soluble drugs show many problems in formulating them in conventional dosage forms. One of the critical problems associated with poorly soluble drugs is too low bioavailability. The problem is even more complex for drugs belonging to BCS CLASS II category, as they are poorly soluble in both aqueous and organic media, and for those drugs having a log P value of 2. There are number of formulation approaches to resolve the problems of low solubility and low bioavailability. These techniques for solubility enhancement have some limitations and hence have limited utility in solubility enhancement. Nanotechnology can be used to resolve the problems associated with these conventional approaches for solubility and bioavailability enhancement. Nanotechnology is defined as the science and engineering carried out in the nanoscale that is 10^{-9} meters. The present article describes the details about nanosuspensions. Nanosuspensions consist of the pure poorly water-soluble drug without any matrix material suspended in dispersion. The review article includes the methods of preparation with their advantages and disadvantages, characterization and evaluation parameters and pharmaceutical applications. A nanosuspension not only solves the problems of poor solubility and bioavailability but also alters the pharmacokinetics of drug and thus improves drug safety and efficacy.

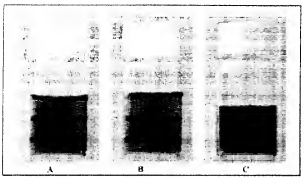
INTRODUCTION: Most of the new chemical entities (about 40%) coming out from High-throughput screening in drug discovery process are failing due to their poor solubility in the water [1]. As per a recent report [2], 46% of the total New Drug Applications (NDA) filed between 1995 and 2002 were BCS class IV, while only 9% were BCS class I drugs, revealing that a majority of the approved new drugs were water insoluble Because of their poor solubility it will become more complicated to incorporate them into the conventional dosage forms and thus decreasing the bioavailability of the drugs [3].

The problem is even more complex for drugs such as Glibenclamide (belonging to BCS CLASS II) as classified by BCS System [4] as they are poorly soluble in both aqueous and organic media, and for those drugs having a log P value of 2. For class II drugs, the rate limiting factor in their intestinal absorption is dissolution/solubility and thus the performance of these drugs is dissolution rate-limited and is affected by the fed/fasted state of the patient. Dissolution rates of sparingly soluble drugs are greatly affected by the shape as well as the particle size of the drug. Therefore decrease in particle size results in an increase in dissolution rate [5]. There are number of formulation approaches that can be used to resolve the problems associated with the low solubility and low bioavailability of these class II drugs. Some of the approaches to increase solubility include micronization [6], solubilisation using co-solvents, use of permeation enhancers, oily solutions, surfactant dispersions [6], salt formation [7] and precipitation techniques [8-9].

Most of these techniques for solubility enhancement have advantages as well as some limitations and hence have limited utility in solubility enhancement. Other techniques used for solubility enhancement like microspheres, emulsions, microemulsions [10], liposomes [11], super critical processing, solid-dispersions [12] and inclusion complexes using Cyclodextrins [13] show reasonable success but they lack in universal applicability to all drugs. These techniques are not applicable to the drugs, which are not soluble in both aqueous and organic Media.

However, there still remains an unmet need to equip the pharmaceutical industry with particle engineering technologies capable of formulating the poorly soluble drugs to improve their efficacy and to optimize therapy with respect to pharmacoeconomics. One such novel technology is nanosuspension technology. Nanosuspensions are sub-micron colloidal dispersions of nanosized drug particles stabilized by surfactants [14]. Nanosuspensions consist of the poorly water-soluble drug without any matrix material suspended in dispersion [15]. These can be used to enhance the solubility of drugs that are poorly soluble in aqueous as well as lipid media. As a result of increased solubility, the rate of flooding of the active compound increases and the maximum plasma level is reached faster.

This is one of the unique advantages that it has over other approaches for enhancing solubility. This approach is useful for molecules with poor solubility, poor permeability or both, which poses a significant challenge for the formulators. The reduced particle size renders the possibility of intravenous administration of poorly soluble drugs without any blockade of the blood capillaries. The suspensions can also be lyophilised and into a solid matrix. Apart from these advantages it is also having the advantages of liquid formulations over others. In the present review we are mainly focussing on the different methods of preparation, critical parameters and evaluation of the nanosuspension. Fig. 1 shows some of the nanosuspensions [16].



A- Gold nanosuspension in water, B -Silver nanosuspension in water, C -VOPc (vanadyl phthalocyanine) nanosuspension in water

FIGURE 1: FEW TYPES OF NANOSUSPENSIONS.

Nanosuspensions differ from nanoparticles [17] which are polymeric colloidal carriers of drugs (Nanospheres and nanocapsules), and from solid-lipid nanoparticles [18] (SLN), which are lipidic carriers of drug. The potential benefits of nanoparticles over conventional technologies are described in **Table 1** [19].

TABLE 1: POTENTIAL BENEFITS OF NANOSUSPENSION TECHNOLOGY

ROUTE OF ADMINISTRATION	POTENTIAL BENEFITS
Oral	• Rapid dissolution and • High bioavailability • Reduced fed/fasted ratio
Intravenous (I.V)	• Tissue targeting • Rapid dissolution • Longer duration of retention in systemic circulation
Ocular	• Higher bioavailability • Less irritation • More consistent dosing
Inhalation	• Higher bioavailability • More consistent dosing
Subcutaneous/ intramuscular	• Higher bioavailability • Rapid onset • Reduced tissue irritation

Preparation of Nanosuspensions: Preparation of nanosuspensions were reported to be a more cost effective and technically more simpler alternative than liposomes and other conventional colloidal drug carriers, particularly for poorly soluble drugs and yield a physically more stable product. The simplest method of preparation of nanosuspensions is micronization by colloid or jet milling [20], which improves the dissolution rate but is not having any effect on saturation solubility. Nanosuspension engineering processes currently used are preparation by precipitation, high pressure homogenization, emulsion and milling techniques. These techniques and the obtained compounds are summarized in **Table 2** and are briefly described in the following sections. Mainly there are two methods for preparation of nanosuspensions. The conventional methods of precipitation are called 'Bottom Up technology'. The 'Top Down Technologies' are the disintegration methods and are preferred over the precipitation methods. These include Media Milling (Nanocrystals), High Pressure Homogenization in water (Dissocubes), High Pressure Homogenization in nonaqueous media (Nanopure) and combination of Precipitation and High-Pressure Homogenization (Nanoedege). Few other techniques used for preparing nanosuspensions are emulsion as templates, microemulsion as templates etc.

- **Precipitation:** The most common method of precipitation used is anti solvent addition method in which the drug is dissolved in an organic solvent and this solution is mixed with a miscible antisolvent. Mixing processes vary considerably. Precipitation has also been coupled with high shear processing. The NANOEDGE process (is a registered trademark of Baxter International Inc. and its subsidiaries) relies on the precipitation of friable materials for subsequent fragmentation under conditions of high shear and/or thermal energy [32].

TABLE 2: SUMMARY OF THE NANOSUSPENSION FORMATION TECHNOLOGIES

Technology	Advantage	Disadvantage	Drug
Precipitation	Simple process. Ease of scale up. Economical production.	Growing of crystals needs to be limit by surfactant addition. Drug must be soluble at least in one solvent.	Carbamazepine [8] Cyclosporine [71] Griseofulvin [72]
Emulsion/Microemulsion template	High drug solubilization. Long shelf life. Ease of manufacture.	Use of high amount of surfactant and stabilizers. Use of hazardous solvent in production.	Bravinclapine [72] Griseofulvin [73]
High pressure Homogenization	Applicable to most of the drugs Very dilute as well as highly concentrate nanosuspension can be prepared. Aseptic production possible.	High number of homogenization cycles. Drug should be in micronized state. Possible contamination could occur from metal ions coming off from the walls.	Albendazole [25] Aphidicolin [26] Azithromycin [27] Fenofibrate [28]
Milling methods			
• Media milling	Applicable to the drugs that are poorly soluble in both aqueous and organic media. Little batch to batch variation. High flexibility in handling large quantities of drugs.	Time consuming. Difficult to scale up. Prolonged milling may induce the formation of amorphous & instability.	Cilostazol [29] Danazol [3] Naproxen [1]
• Dry Co-grinding	Easy process and no organic solvent required. Require short grinding time.	Generation of residue of milling media.	Clarithromycin [30] Glibenclamide [31]

This is accomplished by a combination of rapid precipitation and high-pressure homogenization. Rapid addition of a drug solution to an antisolvent leads to sudden super saturation of the mixed solution, and generation of fine crystalline or amorphous solids. Precipitation of an amorphous material may be favored at high super saturation when the solubility of the amorphous state is exceeded. The success of drug nanosuspensions prepared by precipitation techniques has been reported in some journals [32-33].

- **Lipid Emulsion/Microemulsion Template:** Lipid emulsions as templates are applicable for drugs that are soluble in either volatile organic solvents or partially water miscible solvents. In this method the drug will be dissolved in the suitable organic solvent and then emulsified in aqueous phase using suitable surfactants. Then the organic solvent will be slowly evaporated under reduced pressure to form drug particles precipitating in the aqueous phase forming the aqueous suspension of the drug in the required particle size. Then the suspension formed can be diluted suitably to get nanosuspensions [34]. Moreover, microemulsions as templates can produce nanosuspensions. Microemulsions are thermodynamically stable and isotropically clear dispersions of two immiscible liquids such as oil and

water stabilized by an interfacial film of surfactant and co-surfactant. The drug can be either loaded into the internal phase or the pre-formed microemulsion can be saturated with the drug by intimate mixing. Suitable dilution of the microemulsion yields the drug nanosuspension [34]. An example of this technique is the griseofulvin nanosuspension which is prepared by the microemulsion [34]. The advantages of lipid emulsions as templates for nanosuspension formation are that they easy to produce by controlling the emulsion droplet and easy for scale-up. However, the use of organic solvents affects the environment and large amounts of surfactant or stabilizer are required.

- **High Pressure Homogenization:** It is the most widely used method for the preparation of the nanosuspensions of many poorly water soluble drugs [35-37]. Different methods developed based on this principle for preparation of nanosuspensions are *Dissocubes, Nanopure, Nanoedge, Nanojet technology.* In the high pressure homogenization method, the suspension of a drug and surfactant is forced under pressure through a nanosized aperture valve of a high pressure homogenizer.

The principle of this method is based on cavitation in the aqueous phase. The particles cavitations forces are sufficiently high to convert the drug microparticles into nanoparticles. The concern with this method is the need for small sample particles before loading and the fact that many cycles of homogenization are required [38-39]. Figure 2 gives the schematic representation of the high-pressure homogenization process

- DissoCubes technology is an example of this technology developed by R.H. Müller using a piston-gap-type high pressure homogenizer, which was recently released as a patent owned by SkyePharm plc [34]. Scholer *et al.* prepared atovaquone nanosuspensions using this technique.
- Nanopure is suspensions homogenized in water-free media or water mixtures.
- Nanoedge is combination of precipitation and homogenization techniques resulting in smaller particle size and better stability in a shorter time.
- *Nanojet technology*, also called as opposite stream, uses a chamber where a stream of suspension is divided into two or more parts, which colloid with each other at high pressure.

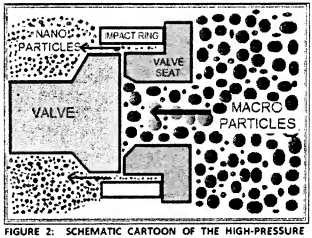


FIGURE 2: SCHEMATIC CARTOON OF THE HIGH-PRESSURE HOMOGENIZATION PROCESS

- **Milling Techniques:**
  - **Media milling:** Media milling is a further technique used to prepare nanosuspensions [24, 40]. This patent-protected technology was developed by Liversidge et al. [41]. Formerly, the technology was owned by the company NanoSystems but recently it has been acquired by Elan Drug Delivery. In this technique, the nanosuspensions are produced using high-shear media mills or pearl mills. The media mill consists of a milling chamber, a milling shaft and a recirculation chamber. The drug nanoparticles are obtained by subjecting the drug to media milling. High energy and shear

forces generated as a result of impaction of the milling media with the drug provide the necessary energy input to disintegrate the microparticulate drug into nanosized particles. The milling medium is usually composed of glass, zirconium oxide or highly cross-linked polystyrene resin. In batch mode, the time required to obtain dispersions with unimodal distribution profiles and mean diameters <200nm is 30–60 min. In the media milling process, the milling chamber is charged with the milling media, water or suitable buffer, drug and stabilizer. Then milling media or pearls are rotated at a very high shear rate.

- **Dry Co-Grinding:** Recently, nanosuspensions can be obtained by dry milling techniques. Successful work in preparing stable nanosuspensions using dry-grinding of poorly soluble drugs with soluble polymers and copolymers after dispersing in a liquid media has been reported [42]. Itoh *et al* [35] reported the colloidal particles formation of many poorly water soluble drugs; griseofulvin, glibenclamide and nifedipine obtained by grinding with polyvinylpyrrolidone (PVP) and sodium dodecylsulfate (SDS).

Many soluble polymers and co-polymers such as PVP, polyethylene glycol (PEG), hydroxypropyl methylcellulose (HPMC) and cyclodextrin derivatives have been used [43]. Physicochemical properties and dissolution of poorly water soluble drugs were improved by co-grinding because of an improvement in the surface polarity and transformation from a crystalline to an amorphous drug [14]. Dry co-grinding can be carried out easily and economically and can be conducted without organic solvents. The co-grinding technique can reduce particles to the submicron level and a stable. Table 3 shows some drugs and their status in market.

TABLE 3: SOME DRUGS AND THEIR STATUS IN MARKET

Drug	Category	Route of Administration	Status
Fenofibrate	Anticancer	Oral	Phase I
Rapamuane	Antiemetic	Oral	Marketed
Emend	Antiasthamatic	Oral	Marketed
Thymectacin	Antidiabetic	I.V.	Phase I/II
Silver	Eczema, Atopic dermatitis	Topical	Phase I
Busulfan	Hypolidemic	Intrathecal	Phase I
Paclitaxel	Anticancer	I. V.	Phase IV
Insulin	Antidiabetic	Oral	Phase I
Budesonide	Anticancer	Pulmonary	Phase I

Physical, Chemical and Biological Properties of Nanosuspensions: Nanosuspension formulation increases the saturation solubility as well as dissolution rate. Basically the saturation solubility is a compound specific constant which is temperature dependent. The saturation solubility also depends on the polymorphism of the drug as different polymorphs have different solubilities. It is also dependent on the particle size. This size-depend encycomes only into effect for particles having a size below approximately 1 µm. Another marked property is the adhesiveness generally described for nanoparticles [45].

As the particle size decreases the adhesive properties of the particles will be improved and thus improved oral delivery of poorly soluble drugs. Improved bioavailability, improved dose proportionality, reduced fed/fasted variability, reduced inter-subject variability and enhanced absorption rate (both human and animal data) [48] are some of the main effects observed on oral administration. These data have been acquired *in vivo* in animals but also in humans as reported by the company Nano Systems. A drastically remarkable report is that of the increase in bioavailability for danazole from 5 % (as macrosuspension) to 82% (as nanosuspension) [46]. The application of high

pressures during the production of nanosuspensions was found to promote the amorphous state [47]. The degree of particle fineness and the fraction of amorphous particles in the nanosuspensions were found to be dependent on production pressure number of cycles of homogenisation and hardness of drug. The increase in the amorphous fraction leads to a further increase of the saturation solubility. The homogenization process (giving uniform particle size) was able to overcome Ostwald ripening [48] which means physical long-term stability as an aqueous suspension [49].

In oral drug administration, the bioavailability mainly depends upon the solubility of the drug, highly active compounds have failed in the past because their poor solubility has limited *in vivo* absorption and did not lead to effective therapeutic concentrations. As an example, Atovaquone is given orally three times 750 mg daily, because of the low absorption of only 10–15%. Oral administration of nanosuspensions can overcome this problem because of the high adhesiveness of drug particles sticking on biological surfaces and prolonging the absorption time.

Evaluation of Nanosuspensions [50-51]: The characterisation of the nanosuspensions is also similar to that of the suspensions such as colour, odour, presence of impurities and other important characteristics as mentioned below.

- *In-Vitro* **Evaluations:**
  - **Particle size and size distribution**
  - **Particle charge (Zeta Potential)**
  - **Crystalline state and morphology**
  - **Saturation solubility and dissolution velocity**
  - **Stability**
- *In-vivo* **evaluation:**
- *In-Vitro* **Evaluations:**

- **Particle size and size distribution:** It is the most important parameter in the evaluation of the suspensions as it is having the direct effect on the solubility and dissolution rate and the physical stability of the formulation. The mean particle size and the width of particle size can be determined by Photon Correlation Spectroscopy (PCS) [52], laser diffraction and coulter current multisizer. Particle size and polydispersity index (PI) governs the saturation solubility, dissolution velocity and biological performance. PCS measures the particle size in the range of 3nm-3 µm only. PI governs the physical stability of nanosuspension and should be as low as possible for long-term stability (Should be close to zero). LD measures volume size distribution and measures particles ranging from 0.05- 80µm upto 2000µm. Atomic Force Microscopy is used for visualization of particle shape [53]. For IV use, particles should be less than 5 µm, considering that the smallest size of the capillaries is 5-6 µm and hence a higher particle size can lead to capillary blockade and embolism.
- **Particle charge (Zeta Potential):** The particle charge is of importance in the study of the stability of the suspensions. Usually the zeta potential of more than ±40mV will be considered to be required for the stabilisation of the dispersions. For electrostatically stabilized nanosuspension a minimum zeta potential of ±30mV is required and in case of combined steric and electrostatic stabilization it should be a minimum of ±20mV of zeta potential is required.
- **Crystalline Sate and Particle Morphology:** It is of importance as there are chances of the polymorphism during the storage of the nanosuspensions. Hence it is necessary to study the crystal morphology of the drug in suspension. Differential Scanning Calorimetry

(DSC) is most commonly used for such studies [54]. When nanosuspensions are prepared drug particles may get converted to amorphous form hence it is essential to measure the extent of amorphous drug generated during the production of nanosuspensions. The X-Ray Diffraction (XRD) is commonly used for determining change in crystallinity and the extent of the amorphous form of drug [55].

- **Saturation solubility and Dissolution Velocity:** The main advantage associated with the nanosuspensions is improved saturation solubility as well as dissolution velocity. These are studied in different physiological solutions at different pH. Kelvin equation and the Ostwald-Freundlich equations can explain increase in saturation solubility. Determination of these parameters is useful to assess *in vivo* performance of the formulation.

- **Stability of Nanosuspensions:** Stability of the suspensions is dependent on the particle size. As the particle size reduces to the nanosize the surface energy of the particles will be increased and they tend to agglomerate. So stabilizers are used which will decrease the chances of Ostwald ripening and improving the stability of the suspension by providing a steric or ionic barrier. Typical examples of stabilizers used in nanosuspensions are cellulosics, poloxamer, polysorbates, lecithin, polyoleate and povidones. Lecithin may be preferred in developing parenteral nanosuspensions [40].

- *In vivo* **evaluation:** The *in vivo* evaluation of the nanosuspensions is specific to drug and route of administration. Most commonly the formulation was given by required route of administration and the plasma drug levels were estimated using HPLC-UV visible Spectrophotometry. Other parameters which are generally evaluated *in vivo* are

  - Surface hydrophilicity/hydrophobicity (determines interaction with cells prior to phagocytosis)
  - Adhesion properties
  - Interaction with body proteins

APPLICATIONS: Formulating the drug as nanosuspensions increases the saturable concentration, dissolution rate as well as bioavailability of the drug. These nanosuspensions are having application in different routes of administrations like oral, parenteral, topical, ophthalmic, mucoadhesive, pulmonary and targeted drug delivery. Oral administration of nanosuspensions is a drug delivery strategy, not only to improve bioavailability, but also to target gastrointestinal bacterial and parasitic infections because of improved adhesion properties. Nanosuspension technology is considered as suitable new colon delivery systems for the treatment of colon cancer, helminth infections, gastrointestinal inflammation or GIT associated diseases like sprue (zoeliaki).

Infections like tuberculosis, listeriosis, leishmaniasis, and toxoplasmosis are caused by parasites residing the macrophages of the MPS, thus being relatively easily accessible by I.V. injected particles. The I.V. injected particles are heavily and quickly taken up by the MPS cells in case they absorb uptake promoting proteins like apolipoproteins. However, some parasites do also reside in the brain (CNS). The brain-localized parasite mostly leads to relapsing infections if not cured. Therefore, it would be of importance to target drug nanoparticles via surface modification to the brain. A successful targeting of the peptide, dalargin, to the brain using Tween 80® surface modified polyisobutylcyanoacrylates nanoparticles has been reported by Kreuter et al. [56]. A nanosuspension of Amphotericin B developed by Kayser *et al.* showed a significant improvement in its oral absorption in comparison with the

conventional commercial formulations [57]. In case of I.V administration the particle size less than 5µm is preferred. The particle size in nano range will favour the passage of the drug particles into the small capillaries in the body without any blockade. A stable intravenously injectable formulation of omeprazole has been prepared to prevent the degradation of orally administered omeprazole [37].

Aqueous suspensions of the drug can be easily nebulised and given by pulmonary route as the particle size is very less. Different types of nebulisers are available for the administration of liquid formulations. Some of the drugs successfully tried with pulmonary route are budesonide, ketotifen, ibuprofen, indomethacin, nifedipine, itraconazole, interleukin-2, p53 gene, leuprolide, doxorubicin etc. [58] Nanosuspensions can be used for targeted delivery also as the surface of the particle can be suitably modified to make them target specific. Kayser formulated a nanosuspension of Aphidicolin to improve drug targeting against leishmania-infected macrophages [26]. Scholer et al. Prapeared a nanosuspension formulation of Atovaquone and showed an improved drug targeting to the brain in the treatment of toxoplasmic encephalitis in a new murine model infected with Toxoplasma gondii [55].

CONCLUSIONS: Nanosuspensions are chiefly seen as vehicles for administering poorly water soluble drugs have been largely solved the dissolution problems to improve drug absorption and bioavailability. Nanosuspension technology can be combined with traditional dosage forms: tablets, capsules, pellets, and can be used for parenteral products. They have recently received increasing attention as colloidal carriers for targeted delivery of various anticancer drugs, photosensitizers, neutron capture therapy agents or diagnostic agents. Because of their submicron size they are easily targeted to the tumour area. Moreover the possibility of surface functionalization with a targeting moiety has open new avenues for targeted delivery of drugs, genes, photosensitizers and other molecules to the desired area. To take advantage of nanosuspension drug delivery, simple formation technologies and variety applications, nanosuspensions will continue to be of interest as oral formulations and non-oral administration develop in the future. It is expected that future research and development work will be carried out in the near future for clinical realization of these targeted delivery vehicle.

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EXHIBIT E

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Physical and chemical stability of drug nanoparticles☆

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ABSTRACT

As nano-sizing is becoming a more common approach for pharmaceutical product development, researchers are taking advantage of the unique inherent properties of nanoparticles for a wide variety of applications. This article reviews the physical and chemical stability of drug nanoparticles, including their mechanisms and corresponding characterization techniques. A few common strategies to overcome stability issues are also discussed.

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Contents

1. Introduction

With significant attention focused on nanoscience and nanotechnology in recent years, nanomaterial-based drug delivery has been propelled to the forefront by researchers from both academia and industry [1–3]. Various nano-structured materials were produced and applied to drug delivery such as nanoparticles [4], nanocapsules [5],

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nanotubes [6], micelles [7], microemulsions [8] and liposomes [9]. In general, the term "nanoparticles" refers to particles with sizes between 1 and 100 nm. However, submicron particles are also commonly referred as nanoparticles in the field of pharmaceutics and medicine [10–14]. Nanoparticles are categorized as nanocrystals [10], polymeric [15], liposomal [9] and solid lipid nanoparticles (SLN) [16] depending on their composition, function and morphology. Given the extensive available literature reviews on SLN, polymeric and liposomal nanoparticles [4,9,15–18], this article will focus only on nanocrystals (pure drug nanoparticles).

The unique nano-scale structure of nanoparticles provides significant increases in surface area to volume ratio which results in notably different behavior, both *in-vitro* and *in-vivo*, as compared to the traditional microparticles [10–12]. Consequently, drug nanocrystals have been extensively used in a variety of dosage forms for different purposes [10,11,14,19,20], such as improving the oral bioavailability of poorly water-soluble drugs by utilizing enhanced solubility and dissolution rate of nanoparticles [21–23]. In the field of pulmonary drug delivery, the nanoparticles are able to deliver the drugs into the deep lungs, which is of great importance for systemically absorbed drugs [11,14]. In addition, injection of poorly water-soluble nanosuspension drugs is an emerging and rapidly growing field that has drawn increasing attention due to its benefits in reducing toxicity and increasing drug efficacy through elimination of co-solvent in the formulation [10,20].

Despite the advantages of drug nanocrystals, they present various drawbacks including complex manufacturing [12,24–26], nanotoxicity [27] and stability issues [10,19,20]. Stability is one of the critical aspects in ensuring safety and efficacy of drug products. In intravenously administered nanosuspensions, for example, formation of larger particles (>5 μm) could lead to capillary blockade and embolism [20], and thus drug particle size and size distribution needs to be closely monitored during storage. The stability issues of drug nanoparticles could arise during manufacturing, storage and shipping. For instance, the high pressure or temperature produced during manufacturing can cause crystallinity change to the drug particles [12,26,28]. Storage and shipping of the drug products may also bring about a variety of stability problems such as sedimentation, agglomeration and crystal growth [29–31]. Therefore, stability issues associated with drug nanocrystals deserve significant attention during pharmaceutical product development. This article reviews existing literature on drug nanoparticle stability, including theory/mechanisms, methods used to tackle the stability problems and characterization techniques, and provides recommendations to improve the current practices. Since the stability issues related to nanoparticle dry powders are usually trivial, this review will only focus on stability of nanosuspensions (drug nanoparticles dispersed in a liquid medium).

2. Stability of drug nanoparticles

2.1. *Effect of dosage form on stability*

The unique characteristics of drug nanoparticles have enabled their extensive application in various dosage forms including oral, parenteral, ocular, pulmonary, dermal and other specialized delivery systems [10,11,13,20,32]. Although different dosage forms may share some common stability issues, such as sedimentation, particle agglomeration or crystal growth, their effects on drug products are quite different. For instance, particle agglomeration could be a major issue in pulmonary drug delivery since it affects deposition amount/site, and thus drug efficacy. On the other hand, agglomeration in intravenous formulations can cause blood capillary blockage and obstruct blood flow. Moreover, the selection of stabilizers is also closely related to dispersion medium, dosage form and strictly governed by FDA regulations. To date, there is a wide variety of choices on the approved stabilizers for oral dosage form whereas the excipients allowed for inhalation are very limited [33].

Drug nanoparticles exist in the final drug products either in dry powder or suspension form. Examples of the dry powder form include the dry powder inhaler, lyophilized powder for injection and oral tablets or capsules. Solid dosage forms usually have good storage stability profiles, which is why a common strategy to enhance nanosuspension stability is to transform the suspension into solid form [19,25]. Most of the reported stability concerns arise from nanosuspensions in which the drug nanoparticles are dispersed in a medium with or without stabilizers. In addition, mechanisms involved in the stability of small and large biomolecule formulations are different due to their molecular structure differences. A small molecule drug is defined as a low molecular weight non-polymeric organic compound while large biomolecules refer to large bioactive molecules such as protein/peptide. One of the major issues with protein/peptide stability is to maintain the 3-dimensional molecular conformation, such as secondary and tertiary structure in order to keep their biological activities [34,35], whereas there is no such concern for small organic molecules.

2.2. *General stability issues related to nanosuspensions*

Stability issues associated with nanosuspensions have been widely investigated and can be categorized as physical and chemical stability. The common physical stability issues include sedimentation/creaming, agglomeration, crystal growth and change of crystallinity state.

2.2.1. *Sedimentation or creaming*

Drug particles can either settle down or cream up in the formulation medium depending on their density relative to the medium. The sedimentation rate is described by Stokes' law [36,37] which indicates the important role of particle size, medium viscosity and density difference between medium and dispersed phase in determining the sedimentation rate. Decreasing particle size is the most common strategy used to reduce particle settling. Matching drug particles density with medium or increasing medium viscosity are the other widely used approaches to alleviate sedimentation problems [37,38]. Fig. 1 shows different sedimentation types that occur in suspension formulations.

In a deflocculated suspension (Fig. 1a), particles settle independently as small size entities resulting in a slow sedimentation rate. However, densely packed sediment, known as caking [39], is usually formed due to the pressure applied on each individual particle. This sediment is very difficult to be re-dispersed by agitation [36,37,39] and would be detrimental to the drug products performance. In the flocculated suspension (Fig. 1b), the agglomerated particles settle as loose aggregates instead of as individual particles [36,37]. The loose aggregates have a larger size compared to the single particle, and thus higher sedimentation rate. The loose structure of the rapidly settling flocs contains a significant amount of entrapped medium and this structure is preserved in the sediment. The final flocculation volume is therefore relatively large and the flocs can be easily broken and re-dispersed by simple agitation. K.P. Johnston et al. [40,41] have recently attempted to achieve stable nanosuspensions via a novel design of flocs structure called "open flocs", as illustrated in Fig. 1c. Thin film freezing was used to produce BSA nanorods with aspect ratio of approximately 24. These BSA nanorods were found to be highly stable when dispersed into hydrofluoroalkane (HFA) propellant, with no apparent sedimentation observed for 1 year. Due to the high aspect ratio of BSA nanorods and relatively strong attractive Van der Waals (VDW) forces at the contact sites between the particles, primary nanorods were locked together rapidly as an open structure upon addition of HFA, inhibiting collapse of the flocs [41]. The low-density open flocs structure was then filled with liquid HFA medium, preventing particle settling. Similar results were shown using needle

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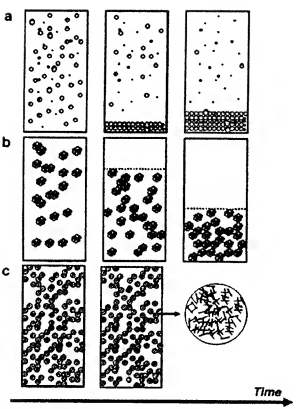

Fig. 1. Sedimentation in (a) deflocculated suspension; (b) flocculated suspension, and (c) open flocs-based suspension.

and plate shaped itraconazole nanoparticles with aspect ratios between 5 and 10 [40].

Although sedimentation is one of the key issues for colloidal suspension, the reported studies examining sedimentation issues in aqueous-based nanosuspensions are very scarce. This could be due to (i) surfactants are generally used in most of the nanosuspensions to inhibit particle agglomeration in the medium, which alleviates the sedimentation issues and (ii) the small nano-sized particles significantly reduce the sedimentation rate. In addition, many of the aqueous nanosuspensions are transformed to dry solid form by spray drying or freeze drying to circumvent the long-term sedimentation issue. Unfortunately, this solidification process cannot be applied to non-aqueous nanosuspensions where sedimentation/creaming is commonly present. An example to illustrate this is metered dose inhaler (MDI) formulations where the nanoparticles are suspended in HFA propellants. Sedimentation or creaming is a key aspect affecting stability of these formulations. Particle engineering to optimize particle surface properties and morphology, e.g. hollow porous particles [42], and introduction of surfactant(s) is generally employed to alleviate the issue.

2.2.2. Agglomeration

The large surface area of nanoparticles creates high total surface energy, which is thermodynamically unfavorable. Accordingly, the particles tend to agglomerate to minimize the surface energy. Agglomeration can cause a variety of issues for nanosuspensions including rapid settling/creaming, crystal growth and inconsistent dosing. The most common strategy to tackle this issue is to introduce stabilizers to the formulation. In addition to safety and regulation considerations, selection of stabilizers is based on their ability to provide wetting to surface of the particles and offer a barrier to prevent nanoparticles from agglomeration [13,19].

There are two main mechanisms through which colloidal suspensions can be stabilized in both aqueous and non-aqueous medium, i.e. electrostatic repulsion and steric stabilization [10,36,37]. These two mechanisms can be achieved by adding ionic and non-ionic stabilizers into the medium, respectively. Stabilization from electrostatic repulsion can be described by the classic Derjagurin–Landau–Verwey–Overbeek (DLVO) theory [43,44]. This theory mainly applies to aqueous suspension while its application in non-aqueous medium is still not well-understood [33]. The DLVO theory assumes that the forces acting on the colloidal particles in a medium include repulsive electrostatic forces and attractive VDW forces. The repulsive forces are originated from the overlapping of electrical double layer (EDL) surrounding the particles in the medium, and thus preventing colloidal agglomeration. The EDL consist of two layers: (i) stern layer composed of counter ion attracted toward the particle surface to maintain electrical neutrality of the system and (ii) Gouy layer which is essentially a diffusion layer of ions (Fig. 2).

The total potential energy (V_T) of particle–particle interaction is a sum of repulsion potential (V_R) generated from electric double layers and attraction potential (V_A) from the VDW forces. V_A is determined by the Hamaker constant, particle size and inter-particulate distance while V_R depends on particle size, distance between the particles, zeta potential, ion concentration and dielectric constant of the medium. V_R is extremely sensitive to ion concentration in the medium. As the ion strength is increased in the medium, the thickness of EDL decreases due to screening of the surface charge [36,37]. This causes decrease in V_R, increasing the susceptibility of the dispersed particles to form aggregates. Zeta potential (ZP) is electric potential at the shear plane which is the boundary of the surrounding liquid layer attached to the moving particles in the medium. ZP is a key parameter widely used to predict suspension stability. The higher the ZP, the more stable the suspension is.

In the case of steric stabilization, amphiphilic non-ionic stabilizers are usually utilized to provide steric stabilization which is dominated by solvation effect. As the non-ionic stabilizers are introduced into nanosuspensions, they are absorbed onto the drug particles through an anchor segment that strongly interacts with the dispersed particles, while the other well-solvated tail segment extends into the bulk medium (Fig. 3).

As two colloidal particles approach each other, the stabilizing segments may interpenetrate, squeezing the bulk medium molecules out of the inter-particulate space as illustrated in Fig. 3. This interpenetration is thermodynamically disfavored when a good solvent is used as the bulk medium to stabilize the tail [36]. Accordingly, provided that the stabilizers can be absorbed onto the particle surface through the anchor segment, strong enthalpic interaction (good solvation) between the solvent and the stabilizing segment of the stabilizer is the key factor to achieve steric stabilization and prevent particles from agglomeration in the medium [36,37]. In addition to solvation, the stabilizing moiety needs to be sufficiently long and dense to maintain a steric barrier that is capable of minimizing particle–particle interaction to a level that the VDW attractive forces are less than the repulsive steric forces [43–45].

The main drawback associated with the steric stabilization is the constant need to tailor the anchoring tail according to the particular drug of interest. Due to the lack of fundamental understanding of interaction between the stabilizers and dispersed nanoparticles, current surfactant screening approaches to achieve a successful steric stabilization are mostly empirical and could be very burdensome [45–49]. In addition, the solvation of the stabilizing segment is susceptible to variation in temperature. Stabilizer concentration could also play a role in causing suspension instability by affecting the absorption affinity of non-ionic stabilizers to drug particles surface. Deng et al.

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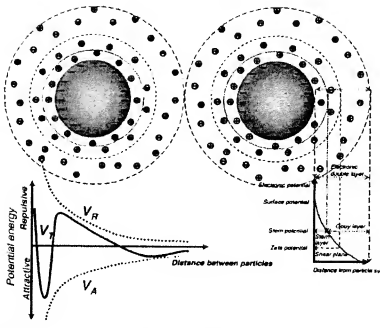

Fig. 2. Illustration of classical DLVO theory. Attractive forces are dominant at very small and large distances, leading to primary and secondary minimum, while repulsive forces are prevailing at intermediate distances and create net repulsion between the dispersed particles, thus preventing particle agglomeration.

[50] used Pluronic® F127 to stabilize paclitaxel nanosuspensions. It was reported that stabilizers had high affinity to nanocrystals surface at concentrations below critical micelle concentration (CMC), and increasing concentrations above CMC caused loss of F127 affinity to the nanocrystals and thus unstable formulation. This was because F127 monomers on the nanocrystals surface started to aggregate with each other to form micelles as the concentration was increased to the CMC level, leading to a lower affinity to the drug crystals. Temperature was also shown to affect the stabilizer affinity to drug crystals. This is expected since the CMC level is dependent on temperature.

It is apparent that combination of the two stabilization mechanisms can be very beneficial in achieving a stable colloidal dispersion. In addition, the combination of a non-ionic stabilizer with an ionic stabilizer reduces the self repulsion between the ionic surfactant molecules, leading to closer packing of the stabilizer molecules [10,51].

Besides the steric and electrostatic stabilization mechanisms, some other stabilization mechanisms have also been reported. Makhlof et al. produced indomethacin (IMC) nanocrystals using the emulsion solvent diffusion technique [52]. The nanoparticles were stabilized using various cyclodextrins (CyDs) without adding any surfactants. The stabilizing effect was attributed to the formation of a CyD network in the aqueous medium via intermolecular interaction of CyD molecules. The network-like structure was believed to prevent aggregation and crystal growth of IMC nanoparticles initially produced from the solvent diffusion process. Similar stabilization mechanism was also observed in another study where budesonide microsuspension was stabilized with hydroxypropyl-beta-cyclodextrin in HFA medium [53]. Another approach to enhance suspension stability that has increasingly been utilized is engineering of particle morphology. One breakthrough in this area was the porous particle

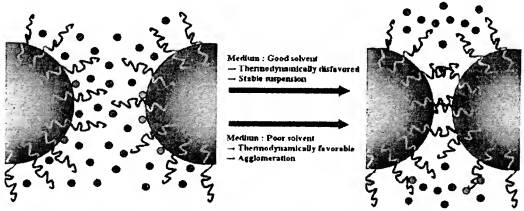

Fig. 3. Steric stabilization mechanisms according to Gibbs free energy: $\Delta G = \Delta H - T\Delta S$. A positive ΔG indicates stable suspension while negative ΔG induces particle agglomeration. If the medium is a good solvent for the stabilizing moiety, the adsorbed stabilizing layers on the dispersed particles cannot interpenetrate each other when the particles collide. This reduces the number of configurations available to the adsorbed stabilizing tails, resulting in a negative entropy change and positive ΔG. On the other hand, if the dispersion medium is a poor solvent, the adsorbed layers on the particles may interpenetrate thermodynamically and induces particles agglomeration.

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concept that was first introduced by Edwards et al. [54]. The porous particles include hollow porous particle [42] and porous nanoparticle-aggregate particles (PNAPs) [14]. Unfortunately, most of the work has been focused on microsuspension or polymeric colloidal formulations and has not been applied to pure drug nanoparticles.

Table 1 summarizes a few published studies on pharmaceutical nanosuspensions. Due to the vast amount of literature work on the pharmaceutical nanosuspensions, this review will focus only on the studies that provide a more profound enlightenment on the stabilizer selection for nanosuspensions. The summary table shows that most of nanosuspensions were generated in aqueous medium, with only a limited number of nanosuspensions made in non-aqueous environment. The commonly used ionic stabilizers in aqueous medium include sodium dodecyl sulfate (SDS), sodium lauryl sulfate (SLS), lecithin and docusate sodium. The non-ionic surfactants used in aqueous medium are usually selected from Pluronic® surfactants, Tween 80, polyethylene glycol (PEG), polyvinyl alcohol (PVA) polyvinylpyrrolidone (PVP) and cellulose polymers such as hydroxypropyl cellulose (HPC) and hydroxypropyl methylcellulose (HPMC).

The stabilizers are not only used to provide short- and long-term storage stability for nanosuspensions, but also to achieve successful formation and stabilization of nanocrystals during particle production. Lee et al. designed and synthesized various amino acid copolymers containing lysine as the hydrophilic segments with alanine, phenylalanine or leucine as hydrophobic moieties [49]. Wet comminution was used to produce naproxen nanosuspensions in presence of HPC and amino acid copolymers. Lysine copolymer with alanine was unable to produce submicron particles while the other copolymers with phenylalanine and leucine were capable of forming the nanoparticles. The size of nanocrystals was proven to be constant over 1 month storage and the crystallinity was also shown to be preserved after the wet comminution process. Furthermore, hydrophobicity of the copolymers was identified as the key factor in achieving the stable nanosuspensions, attributed to strong polymer adsorption onto the hydrophobic drug surfaces. Although this work did not provide an in-depth discussion on how the copolymers interacted with the drug nanoparticles, it illustrated the importance of careful selection of the anchor group (that is attached to the drug surface) in facilitating the production of a stable nanosuspension. In the subsequent study [45], they attempted to understand the nature of interactions between polymeric stabilizers and drugs with different surface energies. Nanocrystals of seven model drugs with PVP K30 and HPC as stabilizers were generated using wet comminution. It was expected that a close match of surface energy between the stabilizers and drug crystals would promote the absorption of stabilizers onto drug particles, and thus help in reducing the particle size during the wet comminution process. Although surface energy did not seem to correlate well with particle size for HPC stabilized system, some trend was observed for PVP stabilized suspension with only one exception.

A further study with seven stabilizers (non-ionic stabilizers: HPC, PVP K30, Pluronic® F127 & F68, PEG and ionic stabilizers: SDS and benzethoinum chloride) and eleven model drugs was conducted by the same group in order to provide more understanding on the stabilization mechanism [48]. Again, the general trend between surface energy and particle size reduction was not observed in this work. PEG was unsuccessful in reducing the particle size of most drug candidates while the other non-ionic stabilizers proved to be effective in reducing the size of five drug candidates that had similar surface energies to the stabilizers. F68 was shown to be the most effective stabilizer (successfully stabilizing nine drug candidates), which could be due to its strong chain adsorption onto the drug crystals through the hydrophobic polypropylene glycol (PPG) units. F127 was found to be less efficient than F68 likely because the short processing time led to inefficient physical adsorption of higher molecular weight F127 to the drug surface. This study demonstrated that a combination of ionic and non-ionic stabilizers is not always beneficial to enhance stabilization. A few combinations of SDS or benzethonium chloride with various non-ionic stabilizers resulted in positive stability effects while the others did not. The effects of physicochemical properties of the drugs on the stabilization were also explored in this study. In general, drugs with lower aqueous solubility, higher molecular weight and higher melting point were shown to have higher chance for successful nanosuspension formation.

Van Eerdenbrugh et al. conducted an expanded study using 13 stabilizers at 3 different concentrations to stabilize 9 drug compounds [47]. The particles were generated using the wet milling technique. The success rate in producing nanosuspensions using polysaccharide based stabilizers [HPMC, methylcellulose (MC), hydroxyethylcellulose (HEC), HPC, carboxymethylcellulose sodium (NaCMC), alginic acid sodium (NaAlg)] was limited by the high viscosity of these polymeric stabilizer solutions. Increasing concentration of these stabilizers did not appear to be helpful. In contrast, the other stabilizers [PVP K30, PVP K90, PVA, Pluronic® F68, polyvinyl alcohol–polyethylene glycol graft copolymer (K-IR), Tween 80 and D-α-tocopherol polyethylene glycol 1000 succinate (TPGS)] did not encounter the viscosity issue. PVA was ineffective in producing the nanosuspension and the success probability of PVP K30, PVP K90, F68 and K-IR is highly dependent on their concentration. Higher concentrations (25 wt.% and 100 wt.%) increased the stabilizing efficacy significantly. Tween 80 and TGPS were proven to be most effective stabilizers. Addition of TGPS (at concentrations >25 wt.%) allowed nanosuspension formation for all tested drug compounds. No correlation was observed between drug physicochemical properties (molecular weight, melting point, log p, solubility and density) and nanosuspension formation success rate. It was demonstrated that surface hydrophobicity of the drug candidates was the driving force for nanoparticles agglomeration, thus lowering the success rate of nanosuspension production.

Mishra et al. explored nanosuspension stability issues during both production and storage [29]. Hesperetin nanosuspensions were produced using HPH with Pluronic® F68, alkyl polyglycoside (Plantacare 2000) and inulin lauryl carbamate (Inutec SP1), or Tween 80 as stabilizers. It was demonstrated that all stabilizers were suitable for successful production of hesperetin nanosuspensions. The size of nanocrystals was dependent on power density applied in the homogenization process and the hardness of the crystals. The effect of stabilizers on the particle size was negligible. Short-term stability over a period of 30 days was examined in order to evaluate the stabilizer efficiency. ZP was measured as a key parameter to predict the stability. In distilled water, the ZP values of all the nanosuspensions fell between −30 and −50 mV and the values dropped significantly in the original dispersion medium. This can be explained by the fact that adsorbed layers of large molecules shifted the shear plane to a longer distance from the particle surface, thus reducing the measured value of zeta potential (Fig. 4). However, the low ZP value does not point to an unstable suspension in this case, which could be due to the additional presence of steric stabilization mechanism. Both Inutec and Plantacare stabilized nanosuspensions also showed significant reduction of ZP measured from water to dispersion medium, indicating a thick absorbed steric layer and good stability. F68 exhibited only slight decrease in ZP, indicating a relatively thin stabilization layer. The ZP value of Tween 80 was only −13 mv in the dispersion medium, pointing to a potentially problematic stabilization. The study demonstrated that zeta potential measurement is a good predictor for storage stability. Nanosuspensions stabilized by Inutec and Plantacare were stable at all storage conditions (4, 25 and 40 °C) up to 30 days while F68 stabilized nanosuspensions were shown to be less stable. The Tween 80 formulation stability was the poorest. Pardeike et al. [30] conducted a similar study using phospholipase A2 inhibitor PX-18 nanosuspensions produced by HPH with Tween 80 as stabilizer. In this work, ZP of the homogenized nanosuspensions was dropped from −50 mV to

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Table 1
Literature summary of pharmaceutical nanosuspensions

Nanoparticles compound	Manufacturing technique	Delivery route	Dispersion medium	Stabilizers	Reference
Diridonin	HPH	NA	Water	PVP K25, Brij 78, SDS, Pluronic® F68, lecithin	Gao et al. (2007) [55]
Diridonin	HPH	IV	Water	Pluronic® F68, lecithin	Gao et al. (2008) [56]
Budesonide	HPH	Inhalation	Water	Lecithin, Span 85, tyloxapol, cetyl alcohol	Jacobs et al. (2002) [57]
Buparvaquone	HPH	Inhalation	Water	Pluronic® F68 and PVA	Hernández-Trejo et al. (2005) [58]
Buparvaquone	HPH	Oral	Water	Pluronic® F68 and lecithin	Jacobs et al. (2002) [59]
Diclofenac acid	HPH	Oral	Water	Pluronic® F68	Liu et al. (2008) [60]
Azorhromycin	HPH	NA	Water	Lecithin, Pluronic® F68, Tween 80	Zhang et al. (2007) [61]
Rutin	HPH	Oral	Water	SDS	Mauludin et al. (2009) [62]
Rutin	HPH	Oral	Water	SDS, Tween 80, Pluronic® F68, PVA	Mauludin et al. (2009) [63]
Tarazepide	HPH	NA	Water	Tween 80, Pluronic® F68	Jacobs et al. (2000) [64]
Omeprazole	HPH	IV	Water	Pluronic® F68	Moschwitzer, (2004) [65]
Amphotericin B	HPH	Oral	Water	Tween 80, Pluronic® F68	Kayser et al. (2003) [22]
Nimodipine	HPH	IV	Water	Pluronic® F68, sodium cholic acid and mannitol	Xiong et al., (2008) [66]
Albendazole	HPH	Oral	Water	SLS, Carbopol, PS 80, hpmc	Kumar et al. (2008) [23]
RMKP 22	HPH	NA	Water	Phospholipon 90	Peters et al. (1999) [67]
Hesperetin	HPH	Dermal	Water	Pluronic® F68, Imutec SP1, Tween 80 and Plantacare 2000	Mishra et al. (2009) [29]
Hydrocortisone, prednisolone and dexamethasone	HPH	Opthalmic	Water	Pluronic® F68	Kassem et al. (2007) [68]
Ascorbyl palmitate	HPH	NA	Water	SDS, Tween 80	Teeranachaideekul et al. (2008) [69]
RMKK99	HPH	NA	Water	Potassium oleate, Tween 80	Krause et al. (2001) [70]
Nifedipine	HPH	NA	Water	HPMC	Hecq et al. (2005) [71]
Undisclosed	HPH	Oral	Water	SLS, HPMC, PVA, Acaciae Gum, Pluronic® F127	Hecq et al. (2006) [72]
Hydroxypropylmethoch	HPH	NA	Water	Lipoid S75, Pluronic® F68, Solutol® H5 15	Zhao et al. (2010) [73]
Azulacrine	HPH	IV	Water	Pluronic® F68	Ganta et al. (2009) [74]
RMKP 22	HPH	NA	Water	Tween 80	Muller et al. (1998) [75]
RMKP 22	HPH	NA	Water	Tween 80, Glycerol	Grau et al. (2000) [76]
PX-18	HPH	NA	Water	Tween 80	Pardeike et al. (2010) [30]
PX-18	HPH	NA	Water	Tween 80	Wang et al. (2010) [77]
Silybin	HPH	Oral, IV	Water	Lecithin, Poloxamer 188	Wang et al. (2010) [78]
Tarazepide	HPH	IV	Water	Pluronic® F68, Tween 80, Glycerol	Jacobs et al. (2000) [64]
Omeprazole, albendazole and danazol	Wet milling	Oral	Water	Pluronic® F108, F68	Tanaka et al. (2009) [79]
Fluticasone, budesonide	Wet milling	Inhalation	Water	Tween 80	Yang et al. (2008) [80]
Naproxen	Wet milling	NA	Water	HPC, arginie hydrochlonde	Ain- Au et al. (2008) [81]
Loviride	Wet milling	NA	Water	Tween 80, Pluronic® F68	Van Eerdenbrugh et al. (2007) [82]
Nine different compounds	Wet milling	NA	Water	13 different stabilizers	Van Eerdenbrugh et al. (2009) [47]
Zinc Insulin	Wet milling	NA	Water	Pluronic® F68, sodium deoxycholate	Menkio-Liversidge et al. (2004) [83]
Ethyl Diatrizoate	Wet milling	NA	Water	Poloxamine 908	Na et al. (1999) [84]
Cinnarizine, itraconazole and phenylbutazone	Wet milling	NA	Water	TPGS 1000	Van Eerdenbrugh et al. (2008) [85]
Nine different compounds	Wet milling	NA	Water	TPGS 1000	Van Eerdenbrugh et al. (2008) [86]
Beclomethasone dipropionate	Wet milling	Inhalation	Water	PVA	Wiedmann et al. (1997) [87]
Rilpivirine	Wet milling	Parenteral	Water	Pluronic® F108, TPGS 1000	Baert et al. (2009) [88]
Undisclosed	Wet milling	NA	Water	Plasdone S-630, docusate sodium	Deng et al. (2008) [89]
Piposulfan, etoposide, camptothecin, paclitaxel	Wet milling	NA	Water	Tween 80, Span 80, Pluronic® F108, F127	Mensko-Liversidge et al. (1996) [90]
Naproxen	Wet comminution	NA	Water	Copolymers of amino acids	Lee et al. (2003) [40]
Seven different compounds	Wet comminution	NA	Water	HPC, PVP	Choi et al. (2005) [45]
Eleven different compounds	Wet comminution	NA	Water	HPC, PVP, PEG, SDS, Pluronic® F68, F127, benzethonium chloride	Lee et al. (2008) [48]
Dihydroartemisinin	Vibrational rod milling	NA	Water	PVP K30, sodium deoxycholate	Chingunpitak et al. (2008) [91]
Probucol	Vibrational rod milling	NA	Water	PVP, SDS	Pongpeerapat et al. (2008) [92]
Ibuprofen	Precipitation, microfluidization	NA	Water	SLS, PVP K30, Pluronic® F68, F127, Tween 80, HPMC	Verma et al. (2009) [31]
Hydrocortisone	Precipitation, microfluidization	NA	Water	PVP, HPMC, SLS	Ali et al. (2009) [93]
Ibuprofen	Solvent diffusion, melt emulsification	NA	Water	PVA, PVP K25, Pluronic® F68, Tween 80,	Kocbek et al. (2006) [94]
Alendronate-gallium, alendronate-gadolinium	Complex precipitation	NA	Water	None	Epstein et al. (2007) [95]
Paclitaxel	Stabilization of nanocrystal (SNC)	NA	Water	Pluronic® F127	Deng et al. (2010) [50]
Felodipine	Antisolvent precipitation & Wet-milling	NA	Water	PVP K30, SDS, docusate sodium	Lindfors (2007) [96]
Naproxen	Antisolvent precipitation	Oral	Water	PVP K15, Pluronic® F127	Chen et al. (2009) [97]
Carbamazepine	Antisolvent precipitation	NA	Water	HPMC, PVP K17	Douroumis et al. (2007) [98]
Cyclosporin A	Antisolvent precipitation	Inhalation	Water	Tween 80	Tam et al. (2008) [99]
Undisclosed	Antisolvent precipitation, Wet milling	IV, Oral	Water	PVP, SDS, Miglyol, docusate sodium	Sigfridsson et al. (2007) [100]
β-methasone valerate-17, oxcarbazepine	Antisolvent precipitation	NA	Water	HPMC, lipoid S75, PEG-5 soy sterol	Douroumis et al. (2006) [101]
Retinoic acid	Antisolvent precipitation	NA	Water	None	Zhang et al. (2008) [102]

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Table 1 (continued)

Nanoparticles compound	Manufacturing technique	Delivery route	Dispersion medium	Stabilizers	Reference
2-devinyl-2-(1-hexyloxyethyl) pyropheophorbide	Antisolvent precipitation	NA	Water	None	Baba et al. (2007) [103]
Nitrendipine	Precipitation–ultrasonication	Oral	Water	PVA	Xia et al. (2010) [104]
Indomethacin	Emulsion diffusion	NA	Water	Cyclodextrins	Makhlof et al. (2008) [52]
Celecoxib	Emulsion diffusion	Oral	Water	Tween 80, PVP K30, SDS	Delenc et al. (2009) [105]
Griseofulvin	Emulsion diffusion	NA	Water	Tween 80, Oramix CG-110	Trotta et al. (2003) [106]
Mitotane	Emulsion diffusion	NA	Water	Tween 80, caprylyl-capryl glucoside, lecithin	Trotta et al. (2001) [107]
Griseofulvin	Microemulsion diffusion	NA	Water	Lecithin	Trotta et al. (2003) [108]
Lysozyme	Emulsification/freeze-drying	Inhalation	HFA	None	Nyamburra et al. (2009) [109]
Bovine serum albumin	Thin film freezing	Inhalation	HFA	None	Engstrom et al. (2009) [41]
Itraconazole	Thin film freezing	Inhalation	HFA	None	Tam et al. (2010) [40]
Insulin	Emulsification + freeze-drying	Inhalation	HFA	Citral, cineole	Nyamburra et al. (2009) [110]
Salbutamol sulfate	Microemulsion + freeze-drying	Inhalation	HFA	Lecithin, docusate sodium	Dickinson et al. (2001) [111]
Salbutamol sulfate	HPH	NA	Acetonitrile	Tween 80	Ahmad et al. (2009) [112]
Horseradish peroxidase, carbonic anhydrase, lysozyme, subtilisin carlsberg and α-chymotrypsin	Freeze-drying	NA	Ethyl acetate	Methyl-β-cyclodextrin	Montalvo et al. (2008) [113]
Diclofenac	Emulsification + freeze drying	Transdermal	Isopropyl myristate	Sucrose ester	Piao et al. (2007) [114]

around −20 mV when tested from water to dispersion medium. It is generally believed that ZP of ±20 mV is sufficient to maintain a stable formulation with a combined electrostatic and steric stabilization [30]. The PX-18 nanosuspension was shown to be physically stable (no changes in particle size distribution) for more than half year at the storage condition of 5 and 25 °C. However, physical instability was observed after 1 month storage at a higher storage temperature. This could be due to the decreased dynamic viscosity and enhanced diffusion constant at higher temperature.

There is another interesting work by Pongpeerapat et al. investigating probucol/PVP/SDS ternary ground mixture (GM) that was prepared with a vibrational rod mill [92]. The produced primary probutcol nanoparticles were around 20 nm in presence of both SDS and PVP. An interesting phenomenon was observed following the dispersion of the GM into water. For GM stabilized with PVP K17 and SDS, spherical agglomerates of primary nanocrystals were formed immediately in the size of around 90 nm after dispersion of the GM into water. A further agglomeration to around 160 nm in size occurred gradually during the storage stability study. In the case of PVP K12 and SDS, agglomerations of approximately 180 nm were observed after 4 days of storage and then remained stable up to 84 days. This phenomenon is illustrated in Fig. 5. Above critical aggregation concentration, SDS complexes with PVP to form a "necklace" structure in aqueous medium through both electrostatic and hydrophobic interactions. Following dispersion of probucol/PVP K17/SDS into water, PVP K17/SDS "necklace" complex interacted with primary drug nanoparticles, causing immediate agglomeration of the primary nanoparticles into 90 nm aggregates. The 160 nm secondary nanoparticles were formed due to further gradual agglomeration process. The stabilization of probucol nanocrystals was attributed to formation of PVP K17/SDS layered structure on the surface of probucol. For the GM of probucol/PVP K12/SDS, agglomeration of primary drug nanoparticles occurred more rapidly because of the insufficient surface coverage of PVP K12 and SDS on the probucol surface. Stabilization of the nanosuspension was linked to absorption of PVP K12 on the surface of probucol nanocrystals, owing to the absence of layered structure.

Despite the proven importance of stabilizers in preventing particle agglomeration, there have been a few studies that generated stable nanosuspensions without stabilizers. Baba et al. prepared 2-devinyl-2-(1-hexyloxyethyl)pyropheophorbide (HPPH) nanosuspensions without any stabilizer and reported formulation stability for more than 3 months [103]. The self-stabilization of the nanosuspensions was attributed to a high ZP value (−40 mv) resulting from the deprotonation of the carboxylic end group of HPPH molecules. A similar self-stabilized nanosuspension was reported in another study in which amorphous all-trans retinoic acid nanoparticles were shown to be stable in aqueous medium up to 6 months. Epstein et al. [95] prepared self-suspended alendronate nanosuspensions by combining the negative charged alendronic acid with gallium (Ga) or

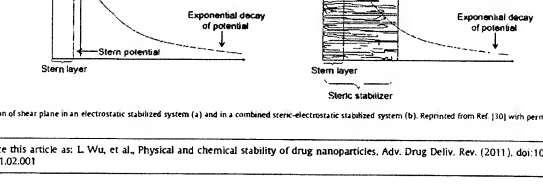

Fig. 4. Location of shear plane in an electrostatic stabilized system (a) and in a combined steric-electrostatic stabilized system (b). Reprinted from Ref. [30] with permission from ELSEVIER.

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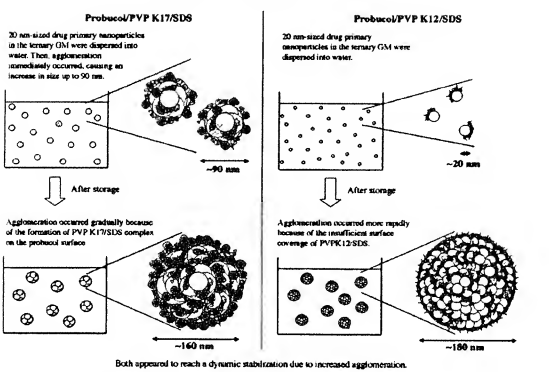

Fig. 5. Schematic overview of agglomeration/stabilization mechanism of probucol/PVP/SDS ternary ground mixture after dispersion into water. Reprinted from Ref. [92] with permission from ELSEVIER.

gadolinium (Gd) under sonication as complex nanoparticles. The alendronate-Ga nanosuspension was shown to be stable for more than 3 months, while the alendronate-Gd nanosuspension was stable for only 3 days. These stability profiles correlated well with their ZP values (33 mV for Ga complex vs. 21 mV for Gd complex).

2.2.3. Crystal growth

Crystal growth in colloidal suspensions is generally known as Ostwald ripening and is responsible for changes in particle size and size distribution. Oswald ripening is originated from particles solubility dependence on their size. Small particles have higher saturation solubility than larger ones according to Ostwald–Freundlich equation [115], creating a drug concentration gradient between the small and large particles. As a consequence, molecules diffuse from the higher concentration surrounding small particles to areas around larger particles with lower drug concentration. This generates supersaturated solution around the large particles, leading to drug crystallization onto the large particles. This diffusion process leaves an unsaturated solution surrounding the small particles, causing dissolution of the drug molecules from the small particles into the bulk medium. This diffusion process continues until all the small particles are dissolved. The Ostwald ripening is essentially a process where large particles grow at the expense of smaller particles [36,37], which subsequently leads to a shift in the particle size and size distribution of the colloidal suspension to a higher range. The diffusion and crystal growth during Ostwald ripening is shown schematically in Fig. 6.

A narrow particle size distribution can minimize the saturation solubility difference and drug concentration gradients within the medium, and thus help to inhibit occurrence of the Ostwald ripening [37]. This can perhaps explain why Ostwald ripening is not a major concern for nanosuspensions with uniform particle size [10,20]. Stabilizers may also alleviate Ostwald ripening as long as they do not enhance the drug solubility [116,117]. Being absorbed on the nanoparticles surface, the stabilizers can reduce the interfacial tension between the solid particles and liquid medium, and thus preventing the Ostwald ripening. Solubility, temperature, and mechanical agitation also affect Ostwald ripening [117]. Verma et al. produced ibuprofen nanosuspensions by microfluidization milling with the aid of various stabilizers (HPMC, Pluronic® F68 & F127, Kollidon 30, SLS) [31]. The particle size obtained with microfluidization showed some correlation with the ibuprofen solubility in aqueous stabilizer solutions. A higher solubility of ibuprofen in the solution of SLS, Tween 80 and Pluronic® F127 resulted in larger particles due to Ostwald ripening that occurred during process. A similar correlation was observed for ibuprofen particles during storage where Ostwald ripening was also believed to the driving factor for formation of larger particles. Van Eerdenbrugh et al. demonstrated that Ostwald ripening was highly dependent on temperature by exploring TPGS stabilized nanosuspensions for 9 different drug candidates [86]. Following 3 months storage at room temperature, Ostwald ripening occurred in 8 out of 9 nanosuspensions studied. Enhanced Ostwald ripening was observed at 40 °C storage, while lowering temperature to 4 °C slowed down or even stopped Ostwald ripening effects.

2.2.4. Change of crystalline state

Crystalline state is one of the most important parameters affecting drug stability, solubility, dissolution and efficacy. The main issue with crystalline state change is the transformation between amorphous and crystalline state. The high energy top-down manufacturing techniques tend to create partially amorphous nanosuspensions and some bottom-up techniques can create completely amorphous particles. The high energy amorphous particles are unstable and inclined to convert to low energy crystalline state over time. This conversion occurs depending on different parameters, such as temperature, dispersion medium, stabilizers and the presence of crystalline particles. Lindfors et al. produced Felodipine amorphous

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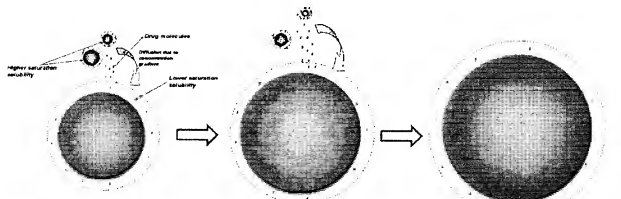

Fig. 6. Schematic illustration of Ostwald ripening.

nanosuspensions via anti-solvent precipitation under sonication [96]. They demonstrated that amorphous nanoparticles were highly unstable in the presence of small amounts of crystalline particles. This was attributed to saturation solubility differences between amorphous and crystalline nanoparticles that initiated a similar diffusion process to Ostwald ripening, leading to a rapid conversion of amorphous nanoparticles to crystalline state. Although most of amorphous particles have been shown to be unstable, a few amorphous nanosuspensions have been demonstrated to be stable over a certain period of time. Amorphous hydrocortisone nanosuspensions, produced through a bottom-up nanoprecipitation technique using microfluidic reactors, was found to remain stable after 3 months storage at room temperature [93]. Amorphous all-trans retinoic acid nanosuspensions, prepared by an anti-solvent precipitation technique, were also shown to be stable over 6 months storage at 4 °C [102].

Manufacturing process might also induce some other type of crystalline transformation. Lai et al. prepared the diclofenac acid (DCF) nanosuspensions by HPH with two different crystalline forms (DCF1 and DCF2) [60]. 5 w/w% Pluronic® F68 was used as a stabilizer. XRD analysis showed that these two crystalline forms belonged to the same polymorph with differences in molecular conformation and crystal size. It was demonstrated that the HPH process caused the partial transformation of DCF2 to DCF1 while no effect on DCF1 was observed. The change in the crystalline structure was attributed to the solubilization of DCF2 during HPH process and its subsequent recrystallization as the DCF1 form.

2.2.5. Stability issues with solidification process of nanosuspensions

When stable nanosuspensions are unattainable, the solid dosage form is the ultimate solution. The most common solidification processes are freeze drying and spray drying [10,19,20,118]. Since most solidified nanoparticle dry powders are usually reconstituted back into nanosuspensions during administration, drug nanocrystal growth or agglomeration during drying process needs to be prevented in order to maintain the nanosizing features such as rapid dissolution following the reconstitution. Adding matrix formers, such as mannitol, sucrose and cellulose, into nanosuspensions prior to drying is the common approach to overcome the stability issues during solidification process [19]. Since several excellent reviews have been published on this topic [19,25,118], the readers are directed to those reviews for more details.

2.2.6. Chemical stability

Since drug nanocrystals are usually dispersed in nanosuspensions with a limited solubility, the possibility of chemical reactions is not as substantial as that in solution-based formulations. Consequently, chemical stability of nanosuspensions is generally superior to that of solutions. Paclitaxel serves as a good example to illustrate this [119]. Fig. 7(a) shows an HPLC diagram of paclitaxel nanosuspensions stabilized with Pluronic® F68 after 4 years of storage at 4–8 °C. No visible degradation product was observed with a recovery of more than 99%. On the other hand, paclitaxel solution with methanol as cosolvent showed clear degradation only after 48 h at room temperature (Fig. 7(b)). The excellent chemical stability of paclitaxel nanosuspensions was attributed to a mechanism similar to oxidized layer on the aluminum surface. Monolayer degradation on the nanocrystals surface was created once they were exposed to water and oxygen, as illustrated in Fig. 7(c). This monolayer could protect the inner part of drug crystals from further degradation, and thus enhance chemical stability of the nanosuspensions.

Unlike the physical stability issue that is a common concern for nanosuspensions, chemical stability is drug specific. Each molecule has its particular functional groups and reaction mechanism that affects the stability. For example, chemical functionalities, such as ester and amides, are susceptible to hydrolytic degradation, while amino groups may undergo oxidative degradation [120]. Although chemical stability of nanosuspensions is usually not a major concern, extra attention should be paid to drug molecules with solubility greater than 1 mg/mL or with low concentration in suspension [120]. The common strategy to enhance the chemical stability is to transform the nanosuspensions into dry solid dosage form which is much more stable than nanosuspensions [19] or to increase the concentration of the nanosuspensions [120].

2.3. Additional stability issues relate to large biomolecules

Large biomolecules discussed in this review are mainly referred to therapeutic protein and peptide. The molecular structure of protein/peptide is distinctly different and more complicated as compared to that of the small molecules. The structures of large molecules are generally differentiated into four structures, i.e. primary, secondary, tertiary and quaternary structures [34]. These different structures refer to the sequence of the different amino acids, regions where the chains are organized into regular local structures by hydrogen bonding such as alpha helix and beta sheet, the mechanisms on how the protein/peptide chain folds into a 3-dimensional conformation, and the composition of multiple protein/peptide molecules assembly, respectively [32,33,123]. The intact molecular structure of protein/peptide is essential to maintain their therapeutic efficacy [35,121]. Common stability issues associated with protein/peptide include deamidation, oxidation, acylation, unfolding, aggregation and adsorption to surfaces [35,121]. These stability issues are affected by

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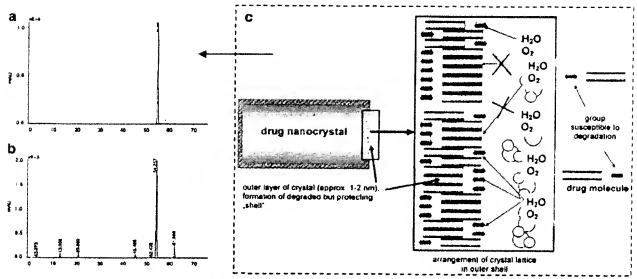

Fig. 7. (a) HPLC diagram of paclitaxel aqueous nanosuspensions stabilized with Pluronic® F68; (b) HPLC diagram of paclitaxel solution (methanol: 10 mL water: 5 mL paclitaxel 20.8 mg); (c) Schematic illustration of stabilization mechanism of paclitaxel nanosuspensions. Reprinted from Ref. [119] with permission from ELSEVIER.

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temperature, solution pH, buffer ion, salt concentration, protein concentration, and added surfactants, with solution formulations being more susceptible to the influence from these factors than the suspension formulations [34,35,121]. Although suspension formulations or solid state of protein/peptide have enhanced stability due to their reduced molecular mobility, other stability issues may arise during particle formation or formulation process. For example, irreversible denaturation and aggregation upon reconstitution were often observed for dehydrated protein through freeze drying or spray-drying [125,126]. To prevent this, supplementary excipients such as bulking agents or surfactants are usually introduced during lyophilization [122].

The vulnerable structure of protein/peptide creates challenges for formulation development. Instead of using "naked" protein, the common strategy to prevent protein/peptide denaturation is to encapsulate the biomolecules with carrier such as liposome [123], SLN [124] or polymeric materials [125,126]. In addition to improving the stability, protein/peptide encapsulation can enhance bioavailability and provide sustained therapeutic release [125–128]. There has been plenty of work reported on encapsulated protein/peptide nanoparticles but very scarce studies on pure protein/peptide nanoparticles. Gomez et al. produced bovine zinc insulin nanoparticles using an electrospray drying technique and reported retained biological activities of the particles [129]. By using HPH, Maschke et al. attempted to micronize insulin in the medium of Myglyol 812 [130]. The stability and bioactivity of the insulin were maintained in spite of the harsh HPH process conditions. Merisko-Liversidge et al. [83] also noticed retained stability and bioactivity of zinc-insulin nanosuspensions that were produced through a wet milling process in presence of Pluronic® F68 and sodium deoxycholate. Nyambura et al. utilized a bottom up technique (combination of emulsification and freeze drying) to generate insulin nanoparticles (80 w/w% insulin with 20 wt.% lactose) [110]. The particles were then dispersed into HFA134a to produce an MDI formulation. The molecular integrity of insulin formulation, measured by HPLC, size exclusion chromatography, circular dichroism and fluorescence spectroscopy, indicated that native structures (primary, secondary and tertiary) were retained after particle formation and formulation process. The presence of surfactant (lecithin) and lyoprotectant (lactose) was believed to be responsible for preservation of the insulin structures. In their follow up work [109], they applied a similar approach to produce composite nanoparticles of lysozyme and lactose for MDI formulations. The retained biological activity of lysozyme was enhanced with increasing lactose concentration in the particles, and reached maximum (99% retained activity) with 20 w/w% lactose. Nanoprecipitation coupled with freeze drying was used as well in this work to produce spherical nanoparticles containing 80 w/w% lysozyme with fully preserved bioactivity. It was demonstrated that bioactivity of lysozyme nanoparticles remained unchanged when in contact with HFA 134a. Yu et al. compared the effectiveness of spray freezing into liquid (SFL) and spray-freeze drying (SFD) processes in producing bioactive lysozyme particles [131]. Both processes generated highly porous micro-sized aggregates of lysozyme primary nanoparticles in the size of 100–300 nm. SFL process was shown to produce lysozyme with lower aggregation and higher enzyme activity as compared to the SFD process, which was attributed to the shorter exposure time to the air–water interface during the SFL atomization process.

3. Characterizing stability of drug nanoparticles and nanoparticle formulations

Selection of characterization techniques for drug nanoparticles stability is dependent on the nature of stability issues and product dosage form. A few commonly used stability characterization techniques are listed in Table 2.

3.1. Particle size, size distribution and morphology

Particle size and size distribution are the key parameters used for evaluating the physical stability of nanoparticles. A variety of techniques, including photon correlation spectroscopy (PCS), also known as dynamic light scattering (DLS), laser diffraction (LD) and coulter counter, are commonly used to measure the particle size and size distribution (Table 2). The PCS/DLS is widely used to determine the size and size distribution of small particles suspended in liquid medium. The mean particle size and size distribution indicated as polydispersity index (PDI) are the typical measured parameters of this technique. A PDI value of 0.1 to 0.25 indicates a narrow size distribution while a PDI greater than 0.5 refers to a broad distribution [20]. Unfortunately, this technique is not capable of measuring size of dry powders and its measurement range is too narrow (3 nm to 3 μm) to detect the interference from the microparticles (>3 μm) within the

Table 2
Commonly used technique to evaluate the stability of nanoparticles.

Measured parameters	Techniques	Remarks
Particle size and size distribution	PCS/DLS	Pros: rapid, non-invasive. Cons: limited measurement range, apply only to liquid suspension
	Laser diffraction	Pros: wide measurement range, rapid, non-invasive, apply to both liquid suspension and dry powder samples. Cons: particles are assumed to be spherical.
	Coulter counter	Pros: precise. Cons: apply only to spherical particles.
Particle size and morphology	SEM/TEM	Pros: evaluate both particle morphology and size, very small quantity of sample required. Cons: challenging to acquire statistical size distribution, usually invasive, time-consuming.
	AFM	Pros: non-invasive, evaluate both particle morphology and size, very small quantity of sample required Cons: challenging to acquire statistical size distribution, time-consuming
Sedimentation/creaming	Visual observation/laser backscattering/ near infrared transmission	-
Particle surface charge/zeta potential	Laser Doppler electrophoresis	-
Crystallinity state	XRD/DSC	-
Chemical stability	HPLC/FTIR/NMR/MS	-

na no suspensions. Therefore, LD is often used in combination with PCS to circumvent this issue. Laser diffraction has a much wider detection range (20 nm to 2000 μm) and it can be used to evaluate both suspension and dry powder samples. The typical LD characterization parameters are LD50, LD90 and LD99, indicating 50, 90 or 99% of the particles are below the given size, respectively. LD is especially suitable for characterizing parenteral and pulmonary suspensions due to it wide measurement range. LD can detect the presence of microparticles (>5 μm) which are detrimental to parenteral nanosuspensions. However, LD provides only relative size distribution. The Coulter counter, on the other hand, measures the absolute number of particles per volume unit for the different size classes, and is more precise than the LD.

Although PCS, LD and coulter counter techniques provide rapid measurement of particle size and size distribution, they do not have the capability in evaluating particle morphology. As direct visualization techniques, Scanning Electron Microscope (SEM), Transmission Electron microscope (TEM) and Atomic Force Microscope (AFM) are widely used for assessment of particle morphology. However, it is very challenging and time-consuming to measure a significant number of particles to achieve statistical size distribution using these techniques. In addition, they usually require additional sample preparation such as coating that could be invasive to the particles, potentially causing some changes in particle properties.

3.2. Sedimentation/creaming

The traditional method to evaluate sedimentation or creaming is by visual observation over a period of time. By measuring the volume of the settled or creamed particle layer relative to the total suspension volume within a specific time, a dimensionless parameter known as sedimentation or flocculation volume can be obtained as a quantitative evaluation of suspension stability. A higher flocculation volume indicates a more stable suspension. The structure of settled/creamed layer can be easily assessed by re-dispersing the suspension, i.e. easily re-dispersed suspension indicates loose flocs while a dense cake is hard to be broken by manual shaking. Other approaches to evaluate sedimentation/creaming include laser backscattering [132] and near-infrared transmission [133].

3.3. Particle surface charge

Laser Doppler electrophoresis is commonly used to measure ZP. This technique evaluates electrophoretic mobility of suspended particles in the medium. It is a general rule of thumb that an absolute value of ZP above 60 mV yields excellent stability, while 30, 20 and less than 5 mV generally results in good stability, acceptable short-term stability and fast particle aggregation, respectively [29]. This rule of thumb is only valid for pure electrostatic stabilization or in combination with low-molecular weight surfactants, and is not valid when higher molecular weight stabilizers are present [29].

3.4. Crystalline state

The crystallinity of drug nanoparticles is usually assessed by X-Ray Diffraction (XRD) and/or Differential Scanning Calorimetry (DSC). XRD differentiates amorphous and crystalline nanoparticles as well as different polymorphic phases of the particles, while DSC is often used as a supplementary tool to XRD. Crystalline particles usually have a sharp melting peak which is absent in amorphous materials. The melting point can also be utilized to differentiate different polymorphs.

3.5. Chemical stability

HPLC is the most common characterization technique used to evaluate chemical stability that provides precise quantitative analysis on the degradation impurities. Mass spectrometry (MS) is often coupled with HPLC to identify the molecular structure of impurities. Some other techniques such as FTIR and NMR can also be used for chemical stability assessment. However, they are not as precise and sensitive as HPLC, and thus not widely used for stability assessment.

3.6. Additional techniques for assessing large biomolecule nanoparticle and formulation stability

For large biomolecules, additional characterization tools are generally required depending on the level of molecular structure to be assessed. For instance, size exclusion chromatography and electrophoresis are used to evaluate the primary structure of large biomolecules, circular dichroism is to monitor the secondary and tertiary structures while fluorescence spectroscopy is for tertiary structure [34,134]. In addition, *in-vitro* bioassays or *in-vivo* efficacy tests are needed to evaluate biological activities of the large biomolecules. Insulin particles, as an example, have been tested for its bioactivity either by *in-vitro* chondrocyte culture assays [130] or *in-vivo* monitoring of blood glucose level on rats following insulin administration [83].

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4. Recommendations of general strategies for enhancing stability of nanoparticle formulations

Strategies to address different stability issues are usually tailored according to different aspects, such as therapeutic requirements, dosage form and manufacturing complexity. For example, as the particle size is reduced, the sedimentation rate is decreased so that the particles can stay suspended longer in nanosuspensions. The general wisdom is that the smaller the nanoparticles are, the better. Unfortunately, too small particles are not always desirable, as they may create undesired plasma peaks due to the significant increases in dissolution rate [28]. Moreover, manufacturing complexity may be increased as well when the particles size requirements become too stringent.

The use of stabilizers is the most commonly used technique in achieving a stable nanoparticle formulation. However, the stabilizer selection is known to be very challenging. The challenge stems mainly from two aspects: (i) lack of fundamental understanding of interactions within nanosuspensions and (ii) lack of an efficient and high throughput stabilizer screening technique. In the case of aqueous nanosuspensions, it is relatively easy to select stabilizers given that water-based stabilizing moieties such as PEG and PVA are well known. However, selecting the anchor groups that interact strongly with the drug surface can be challenging due to the limited understanding on interactions between nanoparticles and stabilizers in molecular level. For non-aqueous nanosuspensions such as HFA-based MDI delivery system, understanding of solvation in the low-dielectric HFA medium is still in its infancy, which makes stabilizers selection even more challenging. Inefficient screening approaches are another hurdle for stabilizer selection. The current practice for stabilizer screening involves trial production of nanosuspensions with different stabilizers or stabilizer combinations, which could be burdensome and require vast amount of efforts especially with a large number of potential stabilizer candidates. AFM has recently been proven to be a feasible and efficient tool for stabilizer screening. Verma et al. demonstrated the feasibility of using AFM to select stabilizers for Ibuprofen nanosuspensions [135]. The AFM measurements showed that HPMC and HPC had extensive surface absorption on the ibuprofen surface, as opposed to the inadequate surface absorption with PVP and Pluronic® surfactants. These results correlated well with their stabilizing performances in the nanosuspensions. This finding confirmed the significance of AFM in providing a scientific rationale for stabilizer selection and improving understanding of the stabilization mechanisms. Another technique, known as colloidal probe microscopy (CPM) which is derived from AFM, has also been widely used to study interactions between colloidal particles and is expected to be a useful tool for nanosuspension stabilizer screening [136].

Due to the significant challenges associated with stabilizer selection, self-stabilized nanosuspensions with no added stabilizer are highly desirable. This is not only for simplifying the formulation development process but also reducing stabilizer-based toxicity. Unfortunately, the challenges to engineer such self-suspended nanoparticles are tremendous with very few reported studies to date. A couple of approaches that could potentially be used to produce self-stabilized nanosuspensions include the creation of drug nanoparticles with high ZP and controlling morphology or surface properties of drug nanoparticles to minimize inter-particulate forces.

5. Conclusions

The stability of drug nanoparticles remains a very challenging issue during pharmaceutical product development. Stability is affected by various factors such as dosage form (nanosuspension vs. dry solid), dispersion medium (aqueous vs. non-aqueous), delivery route (oral, inhalation, IV or other routes), production technique (top-down vs. bottom-up) and nature of drug (small molecules vs. large biomolecules). Despite the significant challenges associated with stabilizer screening, adding a stabilizer or combination of stabilizers is still the most commonly used and preferred approach to enhance the stability of nanosuspensions. Further understanding of particle-particle interactions within nanosuspensions and development of high-throughput stabilizer screening tools are essential to facilitate efficient stabilizer selection. Development of self-stabilized nanosuspensions, although currently seen as very complicated and challenging, is expected to grow with the continuing advancement in the field of particle engineering.

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